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ERRATA AND AUTHORS' EMENDATIONS

Page 41, fifteenth line: "*G*" should be "*I*."

Page 60, table 1, block D, column D, block E, column E, block F, column F, block G, column G, and block H, column H: Last figure "1.0000" should be "—1.0000."

Pages 60 and 64, tables 1 and 9, the three lines of all blocks below the first block are spoken of as line 1, 3, and 4.

Page 61, table 5, block F, column F: Last figure should be "—1.0000."

Page 63, table 8, block I, column G, first line: "0.01456" should be "0.1456"; and block E, column D, fourth line, "1858" should be ".1853."

Page 64, table 9: The brackets in block I, column H, should include the first two lines and the brackets in block H, column H, should include the last three lines.

Page 64, table 9: Instead of beta "AC" in column B insert "AB", and in column A omit "AB."

Page 65, twenty-first line from bottom: "0.944" should be "0.9994."

Page 86, second line: "fig. 3, *C*, *f*" should be "fig. 3, *C*, *b*."

Page 86, fifteenth line: "fig. 4, *C*, *d*" should be "fig. 4, *C*, *e*."

Page 136, legend for plate 1: "*E*" should be "*G*"; "*F*" should be "*E*"; and "*G*" should be "*F*."

Page 137, footnote: Between "percent" and "solution" insert "aqueous" and between "solution" and "to" insert "of phenol."

Page 139, sixteenth line: "pl. 1, *E*" should be "pl. 1, *G*"; twenty-first line, "Plate 1, *F*" should be "Plate 1, *E*"; and thirty-first line, "pl. 1, *G*" should be "pl. 1, *F*."

Page 145, table 1, last column: The cross rule over "Diameter of sorus" should be omitted and the vertical rule at left of column should continue to top.

Page 189, bottom of page: "Key No. G–915" should be "Key No. G–951."

Page 198, line 17: Should be $\frac{\text{Covariance } ws}{\text{Variance } s}$ or $\frac{S(ws-\overline{w}\overline{s})}{S(w-\overline{w})^2}$.

Page 204, fourteenth line: "increase" should be "decrease."

Page 318, ninth line from bottom: "a day's" should be "10 days'."

Page 322, table 20, columns 15 and 16, third-deck boxhead: After "44 days^3" should be "44 days^2."

Page 346, table 10, columns 3 and 6: "$r \pm 0$" should be "$r \neq 0$."

Page 401, table 7, under Victoria (susceptible), second column: "50" should be inserted in blank space.

Page 484, legend for table 1: "Alaska" should be "Alysia" and "Uralde" should be "Uvalde."

Page 550, eleventh line from bottom: Eliminate "an awned fertile lemma."

Page 551, Literature Cited, reference (*j*): "123 pp." should be "23 pp."

Page 607, eleventh line: "(25)" should be "(26)."

Page 741, twenty-second line: "had" should be omitted, and between "germinated" and "at" should be inserted "in the sample taken."

Page 829, ninth line from bottom: Omit "(pl. 2, *C*)."

Page 837, first and second lines: The superscript 6 in the formulas should be a subscript 6.

Page 850, table 2, footnote 2: Should be $\sqrt{\frac{\Sigma x^2}{N} - \bar{x}^2}$.

Page 864, fourteenth line from bottom: "*tritici*" should be inserted after "*graminis*."

Page 868, twelfth line from bottom: "forms" should be "varieties."

JOURNAL OF AGRICULTURAL RESEARCH

VOL. 50 WASHINGTON, D. C., JANUARY 1, 1935 NO. 1

THE UTILIZATION OF ENERGY-PRODUCING NUTRIMENT AND PROTEIN IN WHITE AND YELLOW CORN AND IN DIETS DEFICIENT IN VITAMINS A, D, AND G[1]

By WINFRED W. BRAMAN, *associate professor of animal nutrition;* ALEX BLACK, O. J. KAHLENBERG, and LEROY VORIS, *instructors in animal nutrition;* R. W. SWIFT, *assistant professor of animal nutrition,* and E. B. FORBES, *director, Institute of Animal Nutrition, Pennsylvania Agricultural Experiment Station*

A COMPARISON OF THE NUTRITIVE VALUE OF YELLOW AND WHITE CORN[2]

Many feeding experiments have been conducted for the purpose of comparing the efficiency of white and yellow corn in providing the requirements of maintenance and growth of animals; but in few of these experiments has the composition of the body increase been determined, and in none has there been a complete accounting for the food energy.

REVIEW OF LITERATURE

Steenbock and Boutwell (*32*)[3] compared two rations containing 85 percent of yellow dent corn and 85 percent of white corn, respectively. They found that the yellow corn ration gave from good to normal growth, while the white corn ration failed to promote growth, and in some cases even failed to provide the requirements of maintenance. In the one case in which a white corn ration appeared to produce growth close inspection revealed the presence of some yellow pigment in the white corn.

Steenbock and Coward (*33*) demonstrated the superior value, for growth, of yellow corn over white corn, and also located the vitamin A in the pigment of the endosperm.

Fraps (*8*) compared varieties of corn, as to vitamin A content, in experiments with rats, by Sherman and Munsell's (*29*) unit method, with preliminary depletion of the subjects as to vitamin A, and also by a so-called "ration method", in the use of which there was no preliminary depletion of the rats, the vitamin A being measured by the gain in weight of the animals.

The unit method showed 1 g of yellow corn to contain 2.5 to 8 units per gram, while the vitamin A in most of the samples of white corn was very low—1 unit in 25 to 35 g. Some samples of white corn, however, contained 1 unit in 2 to 5 g of corn—which was considered as possibly resulting from crossing with yellow corn. Season and soil appeared to have some effect on vitamin values.

[1] Received for publication July 25, 1934; issued April 1935. Technical paper no. 648.

[2] The studies of the comparative nutritive value of yellow and white corn were conducted by Braman, Black, Voris, and Forbes; those on the effects of vitamin A deficiency (pp. 13–19) by Braman, Black, and Forbes; those on the effects of vitamin D deficiency (pp. 19–26) by Braman, Black, Swift, and Forbes; and those on the effects of vitamin G deficiency (pp. 26–34) by Swift, Kahlenberg, and Forbes.

[3] Reference is made by number (italic) to Literature Cited, p. 36.

Russell (*26*) found yellow dent corn to be about 50 percent richer in vitamin A than white-capped yellow dent corn.

Hauge, as reported by Skinner (*30*), studied three varieties of corn, Woodburn, with deep yellow endosperm, Reid Yellow Dent, with medium yellow endosperm, and Clement, which contained a smaller amount of yellow. The content of vitamin A varied with the depth of the yellow in these varieties.

Hauge and Trost (*14*), in studies of inheritance of vitamin A in maize, found it to be transmitted exclusively with the yellow endosperm, through the process of crossing and segregation.

Palmer (*23*) and Palmer and Kennedy (*24*) investigated the carotinoids and their relation to growth and reproduction in rats. They concluded that the rat itself contains no carotinoids. Growth and reproduction of rats were maintained on a diet in which the sole source of fat-soluble vitamin was ewe's milk fat containing only 0.00014 percent carotene. Carotinoid-free egg yolk, as the sole source of vitamin A, also served the requirements of growth and reproduction in rats.

Potter (*25*) found that the McIntosh apple, which has white flesh, has a vitamin A potency at least equal to that of two varieties with yellow flesh—Red Delicious and Golden Delicious—and concluded, therefore, that the presence of yellow pigment in the apple is not related to vitamin A potency.

The invariable presence of a yellow pigment with vitamin A, therefore, is not accepted as a fact. Further, the yellow pigment in a feeding stuff is sometimes so located that casual observation does not reveal its presence. The pigment in corn is located in the endosperm, but the outside of the kernel may be white, and may obscure this yellow color. Even when the corn is finely ground the pigment may not be in evidence to superficial inspection.

Kick and Bethke (*15*), in experiments on the effects of storage of corn, found very little if any destruction of vitamin A even when whole, cracked, or finely ground yellow corn was kept for as long as a year.

Practically all of the measurements of vitamin A value, especially by the use of rats, have had as their criterion simply the increase in growth of the animals; but Mitchell and Carman (*21*) went further and studied the utilization of food energy in growing rats and the composition of the gains in weight. They showed that—

> a gain in weight of the live rat, even for a definite age, from a definite initial weight and during a feeding period of definite length, is not a definite measure of the nutritive effect of the food.

Sampson and Korenchevsky (*28*) raised a question as to whether the changes produced in body weight and in the deposition of fat, especially with male rats, on a vitamin A deficient diet, was due to this deficiency alone or to a resulting decrease in appetite and in food consumption. They concluded that vitamin A has both an appetite-producing and an anabolic effect.

The object of the experiment to be discussed was to compare the energy and nitrogen metabolism of rats on two rations of the same gross energy content and of the same gross composition, except that one ration contained yellow and the other white corn.

PRELIMINARY EXPERIMENT

A first trial, of incomplete character, was made with yellow corn and white corn which were purchased locally, but were of unknown varie-

ties and origin. After the corn was ground, the yellow corn meal was decidedly yellow, while the white corn meal was dead white in color.

The composition of the rations was as follows:

YELLOW CORN RATION	*Percent*
Yellow corn	70. 00
Crisco	10. 00
Starch	3. 00
NaCl	1. 00
Osborne and Mendel salts	4. 00
Casein (vitamin-A-free)	12. 00
	100. 00

WHITE CORN RATION	*Percent*
White corn	70. 00
Crisco	10. 80
Gelatin	. 29
Starch	1. 91
NaCl	1. 00
Osborne and Mendel salts	4. 00
Casein (vitamin-A-free)	12. 00
	100. 00

The yellow corn ration contained 2.87 percent N (17.94 percent protein) and 4,276 calories per gram, while the white corn ration contained 2.89 percent N (18.06 percent protein) and 4,375 calories per gram.

Viosterol of a potency of 250 D was added to each ration in the amount of 0.00066 g per 100 g of other components; and one tablet of Harris yeast vitamin (weighing on an average 0.1112 g) was fed daily to each rat.

The casein used was Harris casein, vitamin-B-free. This was washed, to remove vitamin A, by boiling in 95-percent alcohol, for 2 periods of 4 hours each, after which it was dried for 4 days in an oven. The vitamin A content of the casein was then tested by feeding it, in the following diet, to 4 rats: Casein 18 parts, starch 78 parts, Osborne and Mendal salt mixture 4 parts. Viosterol and yeast were fed daily apart from the remainder of the diet.

The experimental animals in the corn-feeding experiment were six pairs of weanling albino rats. The members of each pair were of the same age, litter, and sex, and were of about the same weight (40 g) when weaned at 28 days of age.

The rats were fed by the paired feeding method, as described by Swift, Kahlenberg, Voris, and Forbes (*34*), in the first paper of this series, and use was also made of the same control group of 10 rats, as in the study mentioned, as a basis of computation of the composition of the bodies of the rats at the beginning of this corn-feeding experiment.

One rat of each pair was fed the yellow, and the other the white corn ration. They were fed daily for 13 weeks, the members of each pair of rats receiving the same quantity of feed, and therefore essentially the same quantities of nitrogen and energy.

The rats were kept in individual cylindrical cages, 8 inches in diameter, made of 3-mesh hardware cloth, the cages resting in shallow metal pans. The urine was not saved in this preliminary experiment, but was absorbed by paper toweling in the metal pans. The feces were collected daily, and were analyzed at the end of the experiment as one sample for each rat.

Daily feed records were kept, and the body weight of each rat was recorded every third day. At the end of the 13 weeks' feeding the rats were killed by gas, the body weights were recorded (1) as gross weight, and (2) after the removal of the intestinal contents. The bodies were then dried in vacuum and extracted with ether.[4]

[4] The work of this preliminary experiment, to the time of extraction of the rats, was performed by R. C. Miller, instructor in animal nutrition at the Pennsylvania station.

The data observed from this group of rats were the ether extract and the energy of the ether extract of the rat bodies, the nitrogen and the energy of the ether-extracted bodies, and the nitrogen and the energy of the feces; and, by subtraction of corresponding data from the control group, which has been mentioned, the gains of the rats which received the diets compared were computed.

In the feeding of these rats there were 116 refusals of feed, 75 by the rats on the white corn ration and 41 by the rats on the yellow corn ration. This deviation of 17 from the normal outcome is 3.15 times the standard deviation of 5.39, and would occur by chance only once in 727 trials. This signifies that the yellow corn ration was definitely the more palatable of the two.

After 92 to 93 days' feeding, the rats on the white corn ration alone manifested symptoms of xerophthalmia, and in another day the experiment was terminated. The data obtained from the 6 pairs of rats are shown in tables 1 to 4.

TABLE 1.—*Gain in live weight* [1] *of rats fed yellow corn ration and white corn ration as related to dry matter of food*

Pair no.	Rates fed yellow corn			Rats fed white corn		
	Food eaten (dry matter)	Gain in live weight	Ratio of gain to dry matter of food	Food eaten (dry matter)	Gain in live weight	Ratio of gain to dry matter of food
	Grams	*Grams*	*Percent*	*Grams*	*Grams*	*Percent*
1	539.0	122.0	22.6	546.9	107.0	19.6
2	610.9	196.0	32.1	619.9	159.0	25.6
3	529.1	127.0	24.0	537.8	113.0	21.0
4	457.6	134.0	29.3	463.4	106.0	22.9
5	484.9	114.0	23.5	494.8	100.0	20.2
6	546.1	182.0	33.3	554.1	140.0	25.3
Average	527.9	145.8	27.5	536.2	120.8	22.4

[1] Contents of alimentary tract included.

TABLE 2.—*Energy of body gain of rats fed yellow corn ration and white corn ration as related to energy of food*

Pair no.	Rats fed yellow corn			Rats fed white corn			Body gain of energy of white corn rats as percentage of gain of pair mate
	Food energy	Body gain		Food energy	Body gain		
		Total	As percentage of food energy		Total	As percentage of food energy	
	Calories	*Calories*	*Percent*	*Calories*	*Calories*	*Percent*	*Percent*
1	2,553	364.3	14.3	2,612	253.1	9.7	69.5
2	2,896	544.6	18.8	2,962	463.8	15.7	85.2
3	2,506	377.4	15.1	2,568	337.8	13.2	89.5
5	2,297	416.8	18.1	2,362	342.4	14.5	82.1
6	2,588	460.9	17.8	2,647	426.6	16.1	92.6
Average	2,568	432.8	16.8	2,630	364.7	13.8	83.8

TABLE 3.—*Nitrogen of body gain of rats fed yellow corn ration and white corn ration as related to fat and energy of body gain and to nitrogen of food*

RATS FED YELLOW CORN RATION

Pair no.	Nitrogen of body gain	Fat gained		Energy gained			Nitrogen of food	
		Total	Per gram of nitrogen gained	Total	As fat	As protein	Total	Utilized for body gain
	Grams	*Grams*	*Grams*	*Calories*	*Percent*	*Percent*	*Grams*	*Percent*
1	4.34	24.7	5.7	364.3	61.9	38.1	17.36	25.0
2	6.58	34.4	5.2	544.6	59.0	41.0	19.66	33.5
3	4.12	25.8	6.3	377.4	62.9	37.1	17.04	24.2
4	4.85						14.76	32.9
5	3.59	32.3	9.0	416.8	71.6	28.4	15.64	23.0
6	6.66	27.5	4.1	460.9	54.9	45.1	17.59	37.9
Average	5.02	28.9	6.1	432.8	62.1	37.9	17.01	29.4

RATS FED WHITE CORN RATION

1	4.26	12.5	2.9	253.1	45.8	54.2	17.48	24.4
2	5.99	28.6	4.8	463.8	57.8	42.2	19.79	30.3
3	4.39	21.3	4.9	337.8	59.1	40.9	17.19	25.5
4	4.08						14.83	27.5
5	3.60	23.9	6.6	342.4	65.4	34.6	15.83	22.7
6	4.59	29.4	6.4	426.6	64.1	35.9	17.71	25.9
Average	4.49	23.1	5.1	364.7	58.4	41.6	17.14	26.1

TABLE 4.—*Digestibility of protein and energy-producing nutriment of yellow corn ration and white corn ration when fed to rats*

Pair no.	Rats fed yellow corn						Rats fed white corn					
	Nitrogen			Energy			Nitrogen			Energy		
	Food	Feces	Digested	Food	Feces	Digested	Food	Feces	Digested	Food	Feces	Digested
	Grams	*Grams*	*Percent*	*Calories*	*Calories*	*Percent*	*Grams*	*Grams*	*Percent*	*Calories*	*Calories*	*Percent*
1	17.36	2.64	84.8	2,553	234.5	90.8	17.48	1.32	92.4	2,612	81.0	96.9
2	19.66	2.85	85.5	2,896	270.5	90.7	19.79	1.39	93.0	2,962	90.6	96.9
3	17.04	2.43	85.7	2,506	231.5	90.8	17.19	1.39	91.9	2,568	86.6	96.6
4	14.76	1.74	88.2	2,166	182.3	91.6	14.83	1.19	92.0	2,211	73.2	96.7
5	15.64	2.23	85.7	2,297	192.3	91.6	15.83	1.12	92.9	2,362	69.6	97.1
6	17.59	2.52	85.7	2,588	238.7	90.8	17.71	1.17	93.4	2,647	79.2	97.0
Average	17.01	2.40	85.9	2,501	225.0	91.1	17.14	1.26	92.6	2,560	80.0	96.9

The interpretation of both experiments covered by this paper consists mainly of the comparison of groups of data representing the performance of individual rats on different dietary treatments, these comparisons being made by Love's modification of Student's method (Love (*19*)) of computation of the odds that differences between groups of data are not due to chance alone.

The rats which received the yellow corn ration made greater gains in live weight in all six comparisons than did the rats which received the white corn ration, the average gain of the rats on the yellow corn ration being 145.8 g, as compared with 120.8 g made by the rats on the white corn ration (table 1).

In five comparisons (table 2) a sixth comparison could not be made, since one ether extract was lost; the rats which received the yellow corn ration made more gain in energy than did their pair mates on the white corn ration, the average gains being 432.8 Calories with the yellow as compared with 364.7 Calories with the white corn ration. As to the distribution of this energy gain (table 3) the yellow corn ration was more efficient than the white in storing energy in the form of fat, and the white corn ration was more efficient than the yellow in storing energy in the form of protein.

In all six comparisons (table 4) the rats fed white corn digested more nitrogen and energy than did the rats fed yellow corn the average percentages digested being 85.9 percent nitrogen and 91.1 percent energy for the yellow corn, as compared with 92.6 percent nitrogen and 96.9 percent energy for the white corn ration.

It was noted that with each pair of rats the feces from the individual which received the white corn ration were decidedly darker in color than the feces from its pair mate. Obviously the dark color signified more complete digestion, but the reason for this difference was not apparent. In this connection it is significant that St. Julian and Heller (*27*) found that the digestibility of carbohydrate, protein, and fat are unaffected by a deficiency in vitamin A.

The grams of gain of nitrogen by the bodies of the rats was in favor of the yellow corn ration in 4 comparisons out of 6, 1 other being practically a tie, and 1 in favor of the white corn diet, but statistical criticism indicates that these differences were not certainly significant. There was also a greater percentage utilization of nitrogen in body gain by the rats on the yellow corn ration in 5 out of 6 comparisons. On an average 29.4 percent of the feed nitrogen of the yellow corn ration and 26.1 percent of that of the white corn ration were retained by the body, but the significance of this difference was found to be statistically questionable.

The grams gain of fat by the bodies of the rats was in favor of the yellow corn diet in 4 comparisons out of 5, averaging 28.9 g for the yellow and 23.1 g for the white corn diet, and was statistically significant. The difference in grams of fat per gram of body gain was not significant.

By way of recapitulation, therefore, the yellow corn ration was superior to the white in (1) palatability, (2) gain in weight, (3) gain in energy, and (4) the proportion of the energy gained which was present as fat. On the other hand the white corn ration was superior to the yellow in (1) digestibility of food nitrogen and (2) digestibility of food energy.

No figures were available for the energy utilization, since the urine was not collected.

From this preliminary experiment on yellow and white corn of unknown origin it is clear that the yellow corn used was in important respects superior to the white corn. In this relation the attention naturally directs itself to the difference in vitamin A content, but there is no logical reason for concluding that the observed differences in nutritive value were due solely to this obvious factor—among the many possible differences.

A SECOND EXPERIMENT

A second experiment was conducted, in which corn of known varieties was used. The required quantities of Reid Yellow Dent and Boone County White corn were obtained from a seed merchant in Richmond, Va. After grinding, the meal from the Reid Yellow Dent corn was quite yellow, and that from the Boone County White was "dead white." The rations were:

	Percent		*Percent*
Yellow corn	70. 00	White corn	70. 00
Crisco	10. 18	Crisco	10. 00
Cornstarch	1. 59	Cornstarch	2. 00
NaCl	1. 00	NaCl	1. 00
Osborne and Mendel salts	4. 00	Osborne and Mendel salts	4. 00
Casein (vitamin-A-free)	12. 23	Casein (vitamin-A-free)	12. 00
Irradiated yeast	1. 00	Irradiated yeast	1. 00
	100. 00		100. 00

The casein was a commercial vitamin-free preparation, and the yeast was a dry product of Fleischmann's manufacture containing 12 to 15 Sherman units each of vitamins B and G per gram. This yeast was irradiated and had a potency of 25 D, or 333 Steenbock units, per gram.

The yellow corn ration contained 4,521 calories per gram and 2.814 percent N (17.59 percent protein); and the white corn ration contained 4,528 calories per gram and 2.824 percent N (17.65 percent protein).

Ten pairs of albino rats were used, six pairs of females and four pairs of males. Their ages ranged from 24 to 28 days, and their initial weight from 38.99 to 48.86 g. The members of each pair were of the same litter and sex, and were within 3 g of the same weight. They were fed individually, one rate of each pair receiving the yellow-corn ration and the other the white-corn ration.

During a discussion of methods of feed allotment two procedures other than the paired method were temporarily employed. Thus, during the first 18 days the rats were fed by pairs, in direct proportion to their body weight for the day. During the next 8 days the feed allotment was based on the two-thirds power of a continuing 5-day average weight. Thereafter the regular paired feeding method was followed.

After the paired feeding method was adopted, the quantity of feed given per day was increased until there was refusal of feed by one individual of each pair. At the same time the feeding was so conducted, as to quantities assigned, that the total intake of the rats of each pair was equalized as soon as feasible. Nine of the pairs of rats were fed for 103 to 107 days, and the remaining pair (which were replacements) were fed for 78 days.

The feeding method, and the treatment of the metabolic products, were the same as explained, in some detail by Swift, Kahlenberg, Voris, and Forbes (*34*). Thus the metabolizable energy was computed as the gross energy of the food minus the energy of the urine and feces. Losses of carbon and energy occurring during the process of drying the urine were computed from the loss of nitrogen, on the basis of the assumption that the relationship between the energy and the carbon lost was as in urea.

The total heat production was determined indirectly by a difference procedure, as follows: A control group of rats, representative of the

rats to be fed, was killed at the beginning of the experiment. The alimentary contents were discarded and the bodies were analyzed for nitrogen, fat, and energy. At the end of the feeding experiment all rats were subjected to the same treatment as that given the control group. The body gains of nitrogen, fat, and energy made by the rats which were fed, therefore, were computed as the constituents of these rats minus those of the controls; and the heat production of the rats which were fed was computed as the gross energy of the food minus the energy of the excreta and of the body gain.

There was some coprophagy by rats on both diets. Watching revealed the fact that they would reach back and secure the feces pellets before they had a chance to fall through the screen false bottom of the cage, thus availing themselves of the benefit of the synthetic activities of the organisms of the alimentary tract.

The more important data resulting from this experiment are given in tables 5 to 10.

TABLE 5.—*Gain in live weight* [1] *of rats fed yellow corn ration and white corn ration as related to dry matter of food*

Pair no.	Sex	Rats fed yellow corn			Rats fed white corn		
		Food eaten (dry matter)	Gain in live weight	Ratio of gain to dry matter of food	Food eaten (dry matter)	Gain in live weight	Ratio of gain to dry matter of food
		Gms.	*Gms.*	*Percent*	*Gms.*	*Gms.*	*Percent*
1	♂	691.2	160.61	23.2	692.0	136.06	19.7
2	♂	561.1	147.24	26.2	562.3	136.60	24.3
3	♀	621.4	116.21	18.7	622.6	112.39	18.1
4	♀	667.8	124.18	18.6	669.2	136.46	20.4
5	♂	787.6	170.06	21.6	789.3	150.05	19.0
6	♀	672.8	126.77	18.8	674.3	119.22	17.7
7	♀	687.0	126.05	18.3	688.5	118.20	17.2
8	♀	639.3	120.80	18.9	640.7	106.94	16.7
9	♀	650.5	117.80	18.1	651.9	109.58	16.8
10	♂	441.4	125.20	28.4	442.4	100.50	22.7
Average		642.0	133.49	21.1	643.3	122.60	19.3

[1] Contents of alimentary tract removed.

TABLE 6.—*Energy of body gain of rats fed yellow corn ration and white corn ration as related to energy and to metabolizable energy of food*

Pair no.	Rats fed yellow corn					Rats fed white corn					Body gain of energy of rats fed white corn as percentage of gain of pair mates
	Food energy	Body gain		Metabolizable energy	Body gain as percentage of metabolizable energy	Food energy	Body gain		Metabolizable energy	Body gain as percentage of metabolizable energy	
		Total	Percentage of food energy				Total	Percentage of food energy			
	Calories	*Calories*	*Percent*	*Calories*	*Percent*	*Calories*	*Calories*	*Percent*	*Calories*	*Percent*	*Percent*
1	3,450	388.3	11.3	3,040.6	12.8	3,452	401.7	11.6	3,045.3	13.2	103.5
2	2,801	422.7	15.1	2,437.3	17.3	2,805	403.1	14.4	2,452.2	16.4	95.4
3	3,102	333.0	10.7	2,717.1	12.3	3,106	370.8	11.9	2,722.6	13.6	111.4
4	3,333	375.6	11.3	2,911.2	12.9	3,338	451.3	13.5	2,937.8	15.4	120.2
5	3,931	462.4	11.8	3,425.6	13.5	3,938	573.1	14.6	3,463.0	16.5	123.9
6	3,359	340.5	10.1	2,925.2	11.6	3,364	483.3	14.4	2,960.6	16.3	141.9
7	3,429	370.0	10.8	2,983.5	12.4	3,434	318.7	9.3	3,019.9	10.6	86.1
8	3,191	351.4	11.0	2,822.5	12.4	3,196	287.7	9.0	2,843.1	10.1	81.9
9	3,247	287.2	8.8	2,872.4	10.0	3,252	364.2	11.2	2,869.0	12.7	126.8
10	2,204	314.9	14.3	1,952.1	16.1	2,207	246.2	11.2	1,952.6	12.6	78.2
Average	3,205	364.6	11.5	2,808.7	13.1	3,209	390.0	12.1	2,826.6	13.7	106.9

TABLE 7.—*Heat loss of rats fed yellow-corn ration and white-corn ration as related to energy of food*

Pair no.	Rats fed yellow corn			Rats fed white corn		
	Food energy	Heat loss		Food energy	Heat loss	
		Total	As percentage of food energy		Total	As percentage of food energy
	Calories	*Calories*	*Percent*	*Calories*	*Calories*	*Percent*
1	3,450	2,652.3	76.9	3,452	2,463.6	76.6
2	2,801	2,014.6	71.9	2,805	2,049.1	73.1
3	3,102	2,384.1	76.9	3,106	2,351.8	75.7
4	3,333	2,535.6	76.1	3,338	2,486.5	74.5
5	3,931	2,963.2	75.4	3,938	2,889.9	73.4
6	3,359	2,584.7	76.9	3,364	2,477.3	73.6
7	3,429	2,613.5	76.2	3,434	2,701.2	78.7
8	3,191	2,471.1	77.4	3,196	2,555.4	80.0
9	3,247	2,585.2	79.6	3,252	2,504.8	77.0
10	2,204	1,637.2	74.3	2,207	1,706.4	77.3
Average	3,205	2,444.2	76.2	3,209	2,436.6	76.0

TABLE 8.—*Nitrogen of body gain of rats fed yellow-corn ration and white-corn ration as related to fat and energy of body gain and to nitrogen of food*

RATS FED YELLOW CORN

Pair no.	Nitrogen of body gain	Fat gained		Energy gained			Nitrogen of food	
		Total	Per gram nitrogen gained	Total	As fat	As protein	Total	Utilized for body gain
	Grams	*Grams*	*Grams*	*Calories*	*Percent*	*Percent*	*Grams*	*Percent*
1	5.79	19.3	3.3	388.3	47.2	52.8	21.48	27.0
2	5.37	25.6	4.8	422.7	56.9	43.1	17.43	30.8
3	3.99	20.9	5.2	333.0	59.7	40.3	19.31	20.7
4	4.21	24.7	5.9	375.6	62.0	38.0	20.75	20.3
5	5.93	27.2	4.6	462.4	55.6	44.4	24.47	24.2
6	4.39	19.6	4.5	340.5	54.6	45.4	20.91	21.0
7	4.28	23.8	5.6	370.0	60.3	39.7	21.34	20.1
8	3.90	22.8	5.8	351.4	60.8	39.2	19.86	19.6
9	4.09	15.3	3.7	287.2	50.6	49.4	20.21	20.2
10	4.53	16.4	3.6	314.9	49.5	50.5	13.72	23.0
Average	4.65	21.6	4.7	364.6	55.7	44.3	19.95	23.7

RATS FED WHITE CORN

1	4.83	24.9	5.2	401.7	57.7	42.3	21.53	22.4
2	5.13	24.0	4.7	403.1	56.1	43.9	17.49	29.3
3	3.73	26.1	7.0	370.8	66.4	33.6	19.37	19.3
4	4.37	32.0	7.3	451.3	67.3	32.7	20.82	21.0
5	4.45	44.3	10.0	573.1	73.2	26.8	24.56	18.1
6	3.38	38.7	11.4	483.3	75.4	24.6	20.98	16.1
7	4.02	19.2	4.8	318.7	56.4	43.6	21.42	18.8
8	3.72	16.6	4.5	287.7	54.3	45.7	19.93	18.7
9	3.46	25.3	7.3	364.2	65.3	34.7	20.28	17.1
10	3.69	12.3	3.3	246.2	47.3	52.7	13.76	26.8
Average	4.08	26.3	6.6	390.0	61.9	38.1	20.01	20.8

TABLE 9.—*Urinary carbon of rats fed yellow-corn ration and white-corn ration as related to urinary nitrogen*

Pair no.	Rats fed yellow corn			Rats fed white corn		
	Urinary carbon	Urinary nitrogen	Carbon nitrogen ratio	Urinary carbon	Urinary nitrogen	Carbon nitrogen ratio
	Grams	*Grams*		*Grams*	*Grams*	
1	11.38	12.89	0.9	12.15	13.88	0.9
2	9.72	9.73	1.0	9.90	10.10	1.0
3	10.75	12.43	.9	11.42	12.74	.9
4	10.20	13.60	.8	11.78	13.54	.9
5	13.32	14.43	.9	14.01	16.00	.9
6	11.70	13.72	.9	10.58	14.33	.7
7	12.37	13.13	.9	12.58	14.15	.9
8	10.76	13.25	.8	11.65	13.22	.9
9	10.90	13.34	.8	11.77	13.14	.9
10	6.70	7.59	.9	7.99	7.56	1.1
Average	10.78	12.41	.9	11.38	12.87	.9

TABLE 10.—*Digestibility of protein and energy-producing nutriment of yellow corn ration and white-corn ration when fed to rats*

Pair no.	Rats fed yellow corn						Rats fed white corn					
	Nitrogen			Energy			Nitrogen			Energy		
	Food	Feces	Digested	Food	Feces	Digested	Food	Feces	Digested	Food	Feces	Digested
	Grams	*Grams*	*Percent*	*Calories*	*Calories*	*Percent*	*Grams*	*Grams*	*Percent*	*Calories*	*Calories*	*Percent*
1	21.48	2.09	90.3	3,450	282.2	91.8	21.53	2.03	90.6	3,452	273.8	92.1
2	17.43	1.93	88.9	2,801	261.9	90.6	17.49	1.88	89.3	2,805	248.4	91.1
3	19.31	1.98	89.7	3,102	264.8	91.5	19.37	2.14	89.0	3,106	258.6	91.7
4	20.75	2.18	89.5	3,333	306.5	90.8	20.82	2.11	89.9	3,338	271.5	91.9
5	24.47	2.58	89.5	3,931	363.1	90.8	24.56	2.56	89.6	3,938	323.0	91.8
6	20.91	2.04	90.2	3,359	306.2	90.9	20.98	2.22	89.4	3,364	283.1	91.6
7	21.34	2.64	87.6	3,429	312.2	90.9	21.42	1.88	91.2	3,434	279.2	91.9
8	19.86	1.70	91.4	3,191	253.4	92.1	19.93	1.66	91.7	3,196	222.0	93.1
9	20.21	1.88	90.7	3,247	256.5	92.1	20.28	2.32	88.6	3,252	255.4	92.1
10	13.72	1.11	91.9	2,204	178.3	91.9	13.76	1.25	90.9	2,207	170.8	92.3
Av.	19.95	2.01	90.0	3,205	278.5	91.3	20.01	2.01	90.0	3,209	258.6	92.0

During the entire feeding period there were 308 refusals of feed, 195 by the rats on the white-corn ration and 113 by the rats on the yellow-corn ration. On the basis of these numbers of refusals, without taking into account the quantities of feed refused, the deviation in the 195 refusals is 41 from the normal—154. This is 4.7 times the standard deviation, 8.77, and is therefore significant. This shows that the white corn was definitely the less palatable. It therefore served to determine the intake of both rations.

The data in table 5 shows that, although both members of each pair consumed essentially the same quantity of food, those rats that received the yellow-corn ration gained more weight than did their pair mates in 9 out of 10 comparisons, and the difference was significant. On the basis of the number of differences in weekly gain, which was in favor of the yellow-corn ration, the results are still more definitely significant.

The gain in live weight in relation to dry matter of food necessarily leads to conclusions of the same significance, since both rats of a pair

consumed the same quantity of food. The growth curves representing the gains in weight of the rats are to be found in figure 1, A.

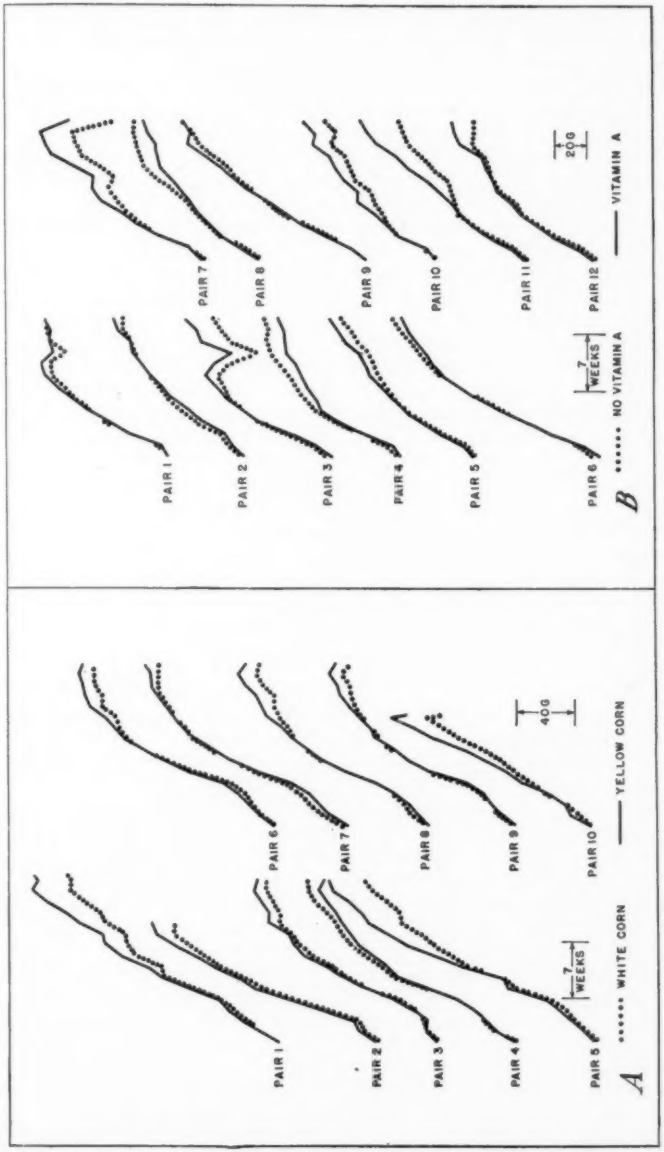

FIGURE 1.—Comparative growth of rats which received: (*A*) Equicaloric quantities of diets containing white and yellow corn; (*B*) equicaloric quantities of a complete diet and of a diet deficient in vitamin A.

In table 8 it is shown that the rats on the yellow-corn diet exceeded their pair mates on the white-corn diet in grams of nitrogen gained,

percentage of food nitrogen retained, and percentage of the energy gain which was present as protein; but it is shown in table 6 that there was no corresponding superiority of the rats fed yellow corn either in amount of energy gained or in efficiency of utilization of metabolizable energy; and in table 8 it is shown that the difference between the two groups in gains in fat and energy were indeterminate. Table 8 shows, however, that with the rats on the white-corn diet a larger proportion of the energy gained was present as fat; and there is somewhat significant evidence that there was more fat gained per gram of nitrogen gained than with the rats on the yellow-corn diet. The greater gain in live weight of the rats on the yellow-corn diet, therefore, consisted of protein and water.

Table 7 shows that there was no significant difference between the two groups as to heat loss.

Table 9 shows that with the rats fed white corn there was somewhat significant evidence of greater outgo of carbon and nitrogen in the urine, but the ratio of carbon to nitrogen in the urine of the two groups was not significantly different.

A difference in the digestibility of the two rations is shown in table 10, in that the energy-producing nutrients of the white-corn diet were slightly but definitely more digestible than were the energy-producing nutrients of the yellow-corn diet; but there was no significant difference in the digestibility of the protein of the two diets.

The ratios of carbon to nitrogen in the urines (table 9) varied between 0.8 and 1.0 for the yellow-corn ration, and between 0.7 and 1.1 for the white-corn ration, the average being 0.9 in both cases. With a low protein ration Swift, Kahlenberg, Voris, and Forbes (*34*) found, in experiments with rats, carbon to nitrogen ratios between 1.6 and 2; and in experiments with steers on a low-protein ration Forbes et al. (*7*) found a urinary carbon to nitrogen ratio as high as 4—these differences apparently being determined, at least in the main, by the plane of protein intake.

No effort was made to deplete the rats as to vitamin A previous to the experiment, and the failure of the rats which received the white-corn diet to manifest xerophthalmia indicates either that they contained a large initial store of this vitamin or that the white-corn diet contained a considerable quantity of this nutrient.

By way of recapitulation it may be said that in this second experiment the yellow-corn diet was shown to be definitely superior to the white-corn diet in the following respects: (1) Palatability, (2) gain in live weight, (3) grams of nitrogen retained, (4) percentage of the energy gained as protein, and (5) percentage of food nitrogen stored. The greater gain in live weight of the rats fed yellow corn consisted of protein and water.

The white-corn diet definitely excelled or exceeded the yellow-corn diet in the following respects: (1) Digestibility of the food energy, (2) percentage of food energy gained as fat, (3) fat gained per gram of nitrogen gained, (4) elimination of carbon in the urine, and (5) elimination of nitrogen in the urine.

The evidence failed to reveal a definite difference in the response of the animals to the two diets in the following respects: (1) Energy of body gain, (2) percentage of metabolizable energy stored, (3) grams of fat gained, (4) heat loss, (5) digestibility of food nitrogen, and (6) the carbon to nitrogen ratio in the urine.

Considering the two experiments together—the yellow-corn ration excelled the white-corn ration in (1) palatability and (2) gain in body weight; while the white-corn ration excelled in digestibility of food energy. In other respects the two experiments—insofar as the same observations were made—were not in definitely significant agreement; but it must be borne in mind that the first experiment covered only a part of the ground covered by the second.

In the interpretation of these results—since they involved a comparison of rations which differed only in the kind of corn—it is necessary to consider the possible influence of the method of food assignment. Thus, the rats which received yellow corn made the larger growth from the same food intake and therefore probably had the larger maintenance requirement—which is mainly for nonnitrogenous nutriment. The rats fed yellow corn, therefore, probably had left available for body increase (after maintenance requirements were satisfied) a smaller quantity and proportion of energy and a larger proportion of nitrogen than did the rats fed white corn. This fact, however, was without observable effect in the results obtained.

Finally, it is necessary to bear in mind the fact that whereas white and yellow corn are well known to differ in vitamin A content, the extent of this difference is far from constant; and it is not known that white and yellow corn differ only in vitamin A content. It must be understood, therefore, that even if it were an established fact (which it is not) that the vitamin A content of corn varies directly as the quantity of yellow-colored substance, there may still be other differences in nutritive value, possibly following other laws of distribution. The color of corn, therefore, while ordinarily indicative of nutritive value, cannot be considered positively significant in this respect.

THE EFFECTS OF VITAMIN A DEFICIENCY

Among the vitamins no other one has been so definitely associated with the phenomenon of growth as vitamin A. The details of this relationship, therefore, are of especial interest, and have been investigated in a growth, metabolism, and body analysis experiment with albino rats as subjects. The procedure as used was the same as that employed by Swift, Kahlenberg, Voris, and Forbes (*34*).

A somewhat similar experiment was conducted by Sampson and Korenchevsky (*28*), who studied the influence of vitamin A deficiency with 63 rats in an experiment in which results of paired and ad libitum feeding were compared. With the performance of the rats fed a complete diet, ad libitum, as the basis of comparison they concluded that two-fifths of the deficiency in the gain in weight of the A-deficient rats was due to deficient food intake, while three-fifths seemed to be due to lack of an anabolic principle; but in relation to fat deposition this relation was reversed—three-fifths of the deficiency being due to decreased food intake and two-fifths to lack of an anabolic principle.

Other papers relating to the effects of vitamin A deficiency on food utilization are reviewed in connection with the foregoing study of the present series.

In consideration of the storage capacity of the animal body for vitamin A the rats used in the present experiment were first subjected to a preparatory depletion of their stores of vitamin A, after which

they were divided into two groups composed of pairs of rats of the same litter, and matched as to sex and weight. One of these groups received a complete basal diet, and the other received a diet differing from the basal one only in that it was deficient in vitamin A. The experimental feeding covered a period of 84 days.

In the management of this experiment important details were the rate of preliminary depletion of the rats as to vitamin A stored within their bodies, and the rate of administration of carotene to the rats on the low-A diet.

Since the conditions of the experiment required that the vitamin-A-deficient rats be kept in a superficially normal condition—at least that they consume enough food to produce growth—and that this would be impossible in the presence of the profound disorganization of fully developed vitamin A deficiency, it was determined to maintain the deficient rats in only moderate vitamin A depletion, and to terminate the experiment as soon as the avitaminosis had become general and acute in the deficient group:

The growth curves (figure 1, *B*) show that the vitamin A deficiency became effective to limit growth at different times with the 12 pairs of rats, and that in general no such influence was apparent during a large part of the experimental period. This resulted mainly from the fact that the well-known characteristic effect of vitamin A deficiency on food intake, and therefore the main effect on growth, was excluded by the maintenance of equicaloric food consumption by pair mates.

The average daily gain in weight of the vitamin-A-deficient rats was 0.96g—which signifies that during the greater part of the experiment the shortage of vitamin A was indeed slight; but a high-grade deficiency would have made it impossible to carry out the plan of the experiment and to maintain equicaloric food intake with both groups of rats without disqualifying hardship to the rats which received the complete diet.

It must be understood, therefore, that the results obtained are representatives only of the particular conditions existing, and that they do not represent long-continued, acute vitamin A deficiency.

The main components of the basal diet were:

	Percent
Casein	18
Osborne and Mendel salt mixture	4
"Amidex" dextrin	68
Crisco	10
	100

In addition, a yeast mixture was fed to both groups of rats, to supply vitamins B, D, and G; and carotene was given regularly to one group, as a source of vitamin A, and to the other group in smaller quantity and only as definite symptoms of vitamin A deficiency were manifest.

The yeast mixture was composed of 57.6 g. of irradiated yeast and 950.4 g. of dried brewer's yeast; and of this a 0.42-g. portion was fed daily to each rat. This supplied 8 Steenbock units of vitamin D, and 5 Sherman units of vitamins B and G. This amount of vitamin D is considered sufficient for normal calcification.

The rats on the complete diet were given 2 International units of vitamin A per day. This was administered as 0.1 cc of a solution of carotene prepared from a carotene-corn-oil solution of a potency of 10,000 International units per gram. The solution as administered was prepared by diluting 0.8 g. of the carotene-corn oil solution with petroleum ether to make 400 cc.

The solution of carotene for the rats on the deficient diet was one-sixteenth the concentration of the solution for the rats on the complete diet, the daily portion of one twenty-fifth cubic centimeter on which they were started containing 0.05 International unit. During the course of the experiment, however, as these rats appeared to require increased amounts of carotene, a solution of four times the concentration stated was prepared, containing 0.1 International unit per one-fiftieth cubic centimeter. Of this more concentrated solution quantities of one-fiftieth cubic centimeter per day or more were administered as required. The more dilute solutions evidently deteriorated, since, during the progress of the experiment, an increasing volume of solution was necessary to maintain a fairly satisfactory food consumption. Nothing was added to this solution to prevent oxidation of the fat in which the carotene was contained. Mattill (*20*) has suggested hydroquinone, among other substances, as an antioxidant for preserving such solutions.

The moderate preliminary depletion of the experimental subjects as to vitamin A was accomplished by feeding the mother rats the vitamin-A-free diet and 8 percent of the yeast mixture mentioned above, from the time the young rats were 2 weeks old until they were weaned at the age of 3 weeks. Foods rich in vitamin A, such as carrots, were withheld during the last 2 weeks of the period of gestation, and milk was fed to the mother rats only during the last week of gestation and thereafter until the young rats were weaned.

In the latter part of the feeding period an attempt was made to detect evidence of the lack of vitamin A by microscopic examination of the vaginal smears, by the technic of Long and Evans (*18*). This observation revealed evidence of the vitamin A deficiency in a number of the rats before it was manifested by any observable external appearance.

The vitamin A deficiency appeared among the 12 rats on the deficient diet after 34 to 55 days. Carotene was then administered from time to time as required to maintain the rats in quasi-normality—that is, on the border line between health and disease, but eating a fair quantity of food.

After 84 days on the experiment the rats which had received carotene regularly were in good condition and showed no signs of lack of vitamin A, either by outward appearance or by the condition of the vaginal smears; but all of their pair mates, which had received the deficient diet, manifested, in various degrees of severity, the well known symptoms of vitamin A deficiency, including the characteristic histology of the vaginal exudate, xerophthalmia, cessation of growth, and lack of normal muscular control in locomotion.

At the end of the feeding experiment all 24 rats were killed, and the bodies, the excreta, and the diets were subjected to chemical analysis. The resulting data are shown in tables 11 to 16.

TABLE 11.—*Gain in live [1] weight of rats fed vitamin-A-supplemented or vitamin-A-deficient diets as related to dry matter of food*

Pair no.	Sex	Rats fed vitamin-A-supplemented diet			Rats fed vitamin-A-deficient diet		
		Food eaten (dry matter)	Gain in empty live weight	Ratio of gain to dry matter of food	Food eaten (dry matter)	Gain in empty live weight	Ratio of gain to dry matter of food
		Grams	*Grams*	*Percent*	*Grams*	*Grams*	*Percent*
1	♀	413.5	77.17	18.7	413.5	77.57	18.8
2	♀	401.8	80.00	19.9	401.8	71.84	17.9
3	♂	407.9	91.00	22.3	406.9	68.74	16.9
4	♀	403.0	73.58	18.3	403.0	82.93	20.6
5	♀	395.3	90.14	22.8	395.3	83.70	21.2
6	♂	492.8	123.10	25.0	492.8	123.04	25.0
7	♂	457.7	79.65	17.4	454.2	50.51	11.1
8	♀	467.7	72.89	15.6	467.7	77.75	16.6
9	♂	519.9	112.57	21.7	519.9	111.93	21.5
10	♀	438.4	81.09	18.5	438.4	66.71	15.2
11	♂	479.9	102.38	21.3	479.9	79.22	16.5
12	♀	519.2	88.47	17.0	519.2	75.08	14.5
Average		449.8	89.34	19.9	449.4	80.75	18.0

[1] Contents of alimentary tract removed.

TABLE 12.—*Energy of body gain of rats on vitamin-A-supplemented or vitamin-A-deficient diets as related to energy and to metabolizable energy of food*

Pair no.	Rats fed vitamin-A-supplemented diet					Rats fed vitamin-A-deficient diet					Body gain of energy of vitamin-A-free rats as percentage of gain of pair mate
	Food energy	Body gain		Metabolizable energy	Body gain as percentage of metabolizable energy	Food energy	Body gain		Metabolizable energy	Body gain as percentage of metabolizable energy	
		Total	As percentage of food energy				Total	Percent of food energy			
	Calories	*Calories*	*Percent*	*Calories*	*Percent*	*Calories*	*Calories*	*Percent*	*Calories*	*Percent*	*Percent*
1	1,848	239.3	12.9	1,687.3	14.2	1,848	252.8	13.7	1,690.4	15.0	105.6
2	1,796	210.6	11.7	1,621.7	13.0	1,796	245.8	13.7	1,619.2	15.2	116.7
3	1,823	232.6	12.8	1,685.8	13.8	1,818	175.3	9.6	1,651.2	10.6	75.4
4	1,801	233.3	13.0	1,658.9	14.1	1,801	275.7	15.3	1,652.0	16.7	118.2
5	1,767	291.3	16.5	1,658.1	17.6	1,767	284.9	16.1	1,619.1	17.6	97.8
6	2,201	320.5	14.6	2,025.9	15.8	2,201	336.9	15.3	2,023.5	16.6	105.1
7	2,045	176.6	8.6	1,879.1	9.4	2,029	76.5	3.8	1,838.7	4.2	43.3
8	2,089	214.5	10.3	1,910.7	11.2	2,089	227.0	10.9	1,918.5	11.8	105.8
9	2,322	295.5	12.7	2,149.9	13.7	2,322	267.6	11.5	2,137.4	12.5	90.6
10	1,959	197.9	10.1	1,799.2	11.0	1,959	167.1	8.5	1,782.3	9.4	84.4
11	2,143	250.6	11.7	1,985.0	12.6	2,143	176.8	8.3	1,952.5	9.1	70.6
12	2,319	244.9	10.6	2,128.4	11.5	2,319	189.5	8.2	2,133.5	8.9	77.4
Average	2,009	242.3	12.1	1,849.2	13.2	2,008	223.0	11.2	1,834.9	12.3	90.9

TABLE 13.—*Heat loss of rats on vitamin-A-supplemented or vitamin-A-deficient diets as related to energy of food*

Pair no.	Rats fed vitamin-A-supplemented diet			Rats fed vitamin-A-deficient diet		
	Food energy	Heat loss		Food energy	Heat loss	
		Total	As percentage of feed energy		Total	As percentage of feed energy
	Calories	*Calories*	*Percent*	*Calories*	*Calories*	*Percent*
1	1,848	1,448.0	78.4	1,848	1,437.6	77.8
2	1,796	1,411.1	78.6	1,796	1,373.4	76.5
3	1,823	1,453.2	79.7	1,818	1,475.9	81.2
4	1,801	1,425.6	79.2	1,801	1,376.3	76.4
5	1,767	1,366.8	77.4	1,767	1,334.2	75.5
6	2,201	1,705.4	77.5	2,201	1,686.6	76.6
7	2,045	1,702.5	83.3	2,029	1,762.2	86.9
8	2,089	1,696.2	81.2	2,089	1,691.5	81.0
9	2,322	1,854.4	79.9	2,322	1,869.8	80.5
10	1,959	1,601.3	81.7	1,959	1,615.2	82.5
11	2,143	1,734.4	80.9	2,143	1,775.7	82.9
12	2,319	1,883.5	81.2	2,319	1,944.0	83.8
Average	2,009	1,606.9	79.9	2,008	1,611.9	80.1

TABLE 14.—*Nitrogen of body gain of rats fed vitamin-A-supplemented or vitamin-A-deficient diets as related to fat and energy of body gain and to nitrogen of food*

RATS FED VITAMIN-A-SUPPLEMENTED DIET

Pair no.	Nitrogen of body gain	Fat gained		Energy gained			Nitrogen of food	
		Total	Per gram of nitrogen gained	Total	As fat	As protein	Total	Utilized for body gain
	Grams	*Grams*	*Grams*	*Calories*	*Percent*	*Percent*	*Grams*	*Percent*
1	2.70	15.7	5.8	239.3	60.9	39.1	13.95	19.4
2	2.43	13.6	5.6	210.6	59.7	40.3	13.62	17.8
3	2.91	14.4	4.9	232.6	57.2	42.8	13.79	21.1
4	2.19	17.3	7.9	233.3	68.4	31.6	13.65	16.0
5	2.76	21.5	7.8	291.3	68.8	31.2	13.43	20.6
6	3.99	19.8	5.0	320.5	57.9	42.1	16.21	24.6
7	2.98	8.3	2.8	176.6	43.8	56.2	15.21	19.6
8	2.32	14.4	6.2	214.5	62.4	37.6	15.50	15.0
9	3.53	18.6	5.3	295.5	58.0	42.0	16.98	20.8
10	2.82	11.2	4.0	197.9	52.2	47.8	14.66	19.2
11	3.31	14.1	4.3	250.6	51.5	48.5	15.84	20.9
12	2.83	16.2	5.7	244.9	61.4	38.6	16.97	16.7
Average	2.90	15.4	5.4	242.3	58.5	41.5	14.98	19.3

RATS FED VITAMIN-A-DEFICIENT DIET

1	2.53	17.5	6.9	252.8	64.1	35.9	13.95	18.1
2	2.13	19.2	9.0	245.8	71.6	28.4	13.62	15.6
3	2.47	9.8	4.0	175.3	51.3	48.7	13.76	18.0
4	2.55	20.2	7.9	275.7	68.6	31.4	13.65	18.7
5	2.36	21.7	9.2	284.9	71.0	29.0	13.43	17.6
6	4.03	21.6	5.4	336.9	59.3	40.7	16.21	24.9
7	2.21	.5	.2	76.5	3.1	96.9	15.11	14.6
8	2.46	15.3	6.2	227.0	63.0	37.0	15.50	15.9
9	3.82	14.9	3.9	267.6	51.6	48.4	16.98	22.5
10	2.37	9.5	4.0	167.1	51.9	48.1	14.66	16.2
11	2.41	10.2	4.2	176.8	53.1	46.9	15.84	15.2
12	2.54	11.2	4.4	189.5	55.2	44.8	16.97	15.0
Average	2.66	14.3	5.4	223.0	55.3	44.7	14.97	17.7

TABLE 15.—*Urinary carbon of rats fed vitamin-A-supplemented or vitamin-A-deficient diets as related to urinary nitrogen*

Pair no.	Rats fed vitamin A			Rats fed vitamin-A-deficient diet		
	Urinary carbon	Urinary nitrogen	Carbon/nitrogen ratio	Urinary carbon	Urinary nitrogen	Carbon/nitrogen ratio
	Grams	*Grams*		*Grams*	*Grams*	
1	5.96	9.63	0.6	6.73	10.03	0.7
2	6.56	9.77	.7	8.18	10.41	.8
3	6.30	9.73	.6	7.03	9.87	.7
4	6.91	10.34	.7	7.10	10.07	.7
5	3.71	6.04	.6	6.23	10.36	.6
6	7.45	11.07	.7	7.84	10.80	.7
7	7.92	11.02	.7	10.03	11.13	.9
8	9.87	11.70	.8	7.11	11.48	.6
9	7.62	11.61	.7	8.05	11.50	.7
10	7.48	10.59	.7	9.91	10.62	.9
11	7.81	11.30	.7	10.60	11.60	.9
12	9.63	12.89	.7	8.56	13.08	.7
Average	7.27	10.47	.7	8.11	10.91	.7

TABLE 16.—*Digestibility of protein and energy-producing nutriment of diets containing or deficient in vitamin A, when fed to rats*

Pair no.	Rats fed vitamin-A-supplemented diet						Rats fed vitamin-A-deficient diet					
	Nitrogen			Energy			Nitrogen			Energy		
	Food	Feces	Digested	Food	Feces	Digested	Food	Feces	Digested	Food	Feces	Digested
	Grams	*Grams*	*Percent*	*Calories*	*Calories*	*Percent*	*Grams*	*Grams*	*Percent*	*Calories*	*Calories*	*Percent*
1	13.95	1.09	92.2	1,848	90.5	95.1	13.95	1.03	92.6	1,848	79.4	95.7
2	13.62	.92	93.2	1,796	96.8	94.6	13.62	.92	93.2	1,796	84.8	95.3
3	13.79	.82	94.1	1,823	63.3	96.5	13.76	1.03	92.5	1,818	87.4	95.2
4	13.65	.75	94.5	1,801	62.5	96.5	13.65	.90	93.4	1,801	68.1	96.2
5	13.43	.93	93.1	1,767	65.7	96.3	13.43	.88	93.4	1,767	74.5	95.8
6	16.21	1.08	93.3	2,201	89.3	95.9	16.21	1.10	93.2	2,201	89.6	95.9
7	15.21	.94	93.8	2,045	75.7	96.3	15.11	1.06	93.0	2,029	80.0	96.1
8	15.50	.94	93.9	2,089	69.2	96.7	15.50	1.13	92.7	2,089	85.8	95.9
9	16.98	1.04	93.9	2,322	82.9	96.4	16.98	1.05	93.8	2,322	90.5	96.1
10	14.66	.99	93.2	1,959	74.0	96.2	14.66	.86	94.1	1,959	67.7	96.5
11	15.84	.92	94.2	2,143	69.1	96.8	15.84	.96	93.9	2,143	73.9	96.6
12	16.97	1.00	94.1	2,319	81.3	96.5	16.97	1.24	92.7	2,319	86.0	96.3
Average	14.98	.95	93.6	2,009	76.7	96.2	14.97	1.01	93.2	2,008	80.6	96.0

In 8 of the 12 pairs of rats, those which received the complete diet made greater gains in live weight than did those which received the vitamin-A-deficient diet, though, in accord with the method of experimentation, both members of each pair consumed the same amount of food. The vitamin-A-deficient rats undoubtedly held back the food consumption and the growth of their pair mates by not consuming as much of the ration as the rats on the complete diet would have eaten. This is shown by the fact that the rats on the vitamin-A-deficient diet refused portions of their food a total of 466 times—as compared with 249 refusals by their pair mates. This is a statistically significant difference.

The growth curves are shown in figure 1, *B*. The average gains in live weight (table 11) of the rats on the complete and on the vitamin-A-deficient diets were 89.34 g and 80.75 g, respectively, this difference being statistically significant.

Table 12 shows that the two groups did not differ in a statistically significant manner with reference to (1) energy of body gain, (2) energy of body gain as a percentage of the food energy, or (3) body gain of energy as a percentage of the metabolizable energy. Similarly, table 13 shows that the heat production was essentially the same for the two groups of rats.

Table 14 shows that the nitrogen storage of the rats on the complete diet was significantly greater than that of their pair mates on the vitamin-A-deficient diet; but there was not a significant difference in the storage of fat, or of fat gained per gram of nitrogen gained, or of total energy gained, or of energy gained as fat or as protein.

The analysis of the urines (table 15) reveals as slightly significant the greater elimination of carbon by the rats on the vitamin-A-deficient diet, but the ratio of carbon to nitrogen in the urine did not differ significantly; nor did the digestibility (table 16) of either the nitrogen or the energy of the diet.

THE EFFECTS OF VITAMIN D DEFICIENCY

Among the various requirements of complete nutrition it is obvious that the different essential nutrients must differ in the nature and the directness of their relationship to the utilization of the protein and the energy of foods; and, in the light of the existing knowledge of the characteristic functions of vitamin D, as essential to the normal utilization of calcium and phosphorus, the relationship of this vitamin to protein and energy metabolism must appear to be indirect and secondary, rather than immediate and essential. However, this relationship may be none the less real for being incidental, and the object of the present experiment was to show the extent and something of the nature of the influence of vitamin D in the connection stated. Since the writers know of no other study of this vitamin made from the point of view of the present investigation, this experiment will be described without general review of the literature on vitamin D.

This experiment consisted of a comparison of two dietary regimens, by means of a growth-, metabolism-, and body-analysis study, on two groups of normal growing rats. One of the diets was known to cause rickets, the other was the same diet plus a vitamin D concentrate.

The rachitogenic diet selected for use in this experiment was Steenbock's diet no. 2965 (*31*), which consisted of yellow corn 76 percent, wheat gluten 20 percent, $CaCO_3$ 3 percent, and NaCl 1 percent.

This diet has been considered by Bacharach, Allchorne, and Hazley (*1*) to be deficient in vitamin A, as well as in vitamin D; but in the study reported in the first section of this paper involving the particular lot of yellow corn used in the present experiment, no evidence of vitamin A deficiency was encountered.

Goldblat (*10*) observed irregular results from the feeding of vitamin-D-free rations containing much $CaCO_3$, apparently due to a disturbance of the ratio of calcium to vitamin D, through the settling out of $CaCO_3$ from the ration. In the present experiment the diets were stored in 2-quart Mason jars, the contents of which were mixed whenever food was removed. The rats did not grow rapidly, but their performance was consistent in the manner of their indicating the presence or the absence of vitamin D.

In this experiment, as in the others of this series, the paired feeding method was used, the subjects being 12 pairs of weanling rats, 27 to 29 days of age, and weighing 42 to 60 g at the beginning of the study.

The individuals of each pair were of the same sex, age, and litter, and of approximately the same body weight. The general routine followed was as in the foregoing studies of this series.

Both rats of each pair received, throughout the experiment, the same quantity of the basal rachitogenic ration specified; but after the first 2 weeks one rat of each pair received in addition 1 vitamin D unit

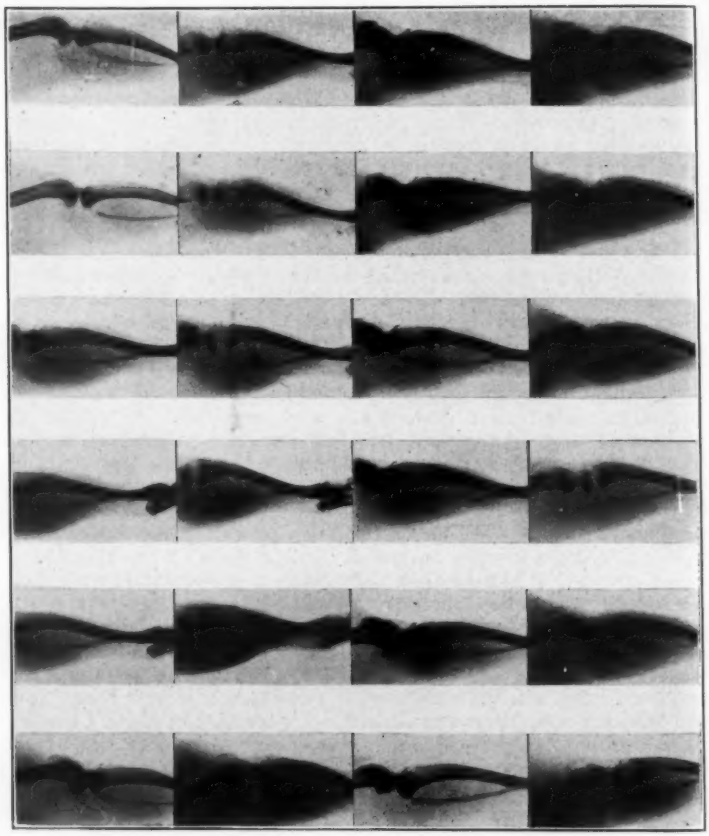

FIGURE 2.—Photographs of leg bones of rats which received a complete diet (*A* and *C*) compared with others which received a vitamin D deficient diet (*B* and *D*). Pairs are arranged in order of number, and pair mates are placed side by side.

(Steenbock) of viosterol (Squibb) each day. For 3 weeks the viosterol was fed in petroleum ether dropped on top of the food, the ether being assumed to have evaporated before the food was offered. Three weeks' experience, however, showed that the petroleum ether did not entirely evaporate, and that it unfavorably affected the palatability of the food. Thereafter the viosterol was fed in olive oil, and the rats which received no viosterol were given olive oil in the same

amounts as were their pair mates. The olive oil improved the appetite of the rats. The refusals of food to the time of beginning the use of olive oil were nearly alike in number for the two groups, but thereafter the number of refusals was significantly less with the rats which received the complete diet.

The feeding period lasted 10 weeks. All of the rats on the basal diet manifested symptoms of vitamin D deficiency, while those which received vitamin D were normal. X-ray photographs of one hind leg of each animal are shown in Figure 2. None of the animals showed any noticeable symptoms of vitamin A deficiency.

In the daily care of the rats the feed-cup holders were removed from the cages in the morning and were not returned until feeding time in the afternoon. This prevented the rats from depositing urine and feces in them.

As soon as the rats on the deficient diet developed noticeable signs of rickets, such as lagging with the hind legs, they and their pair mates were killed, and an X-ray photograph was taken of one hind leg of each individual. The bodies were then analyzed as in the foregoing studies, and the experimental data, given in tables 17 to 22 were statistically interpreted.

TABLE 17.—*Gain in live weight* [1] *of rats fed vitamin-D-supplemented or vitamin-D-deficient diets as related to dry matter of feed*

Pair no.	Sex	Rats fed vitamin-D-supplemented diet			Rats fed vitamin-D-deficient diet		
		Food eaten (dry-matter)	Gain in empty live weight	Ratio of gain to dry matter of food	Food eaten (dry matter)	Gain in empty live weight	Ratio of gain to dry matter of food
		Grams	*Grams*	*Percent*	*Grams*	*Grams*	*Percent*
1	♀	274.2	34.10	12.4	274.2	33.86	12.3
2	♀	312.6	45.58	14.6	312.6	33.30	10.7
3	♂	292.9	44.46	15.2	292.9	36.16	12.3
4	♂	326.2	38.44	11.8	326.2	37.31	11.4
5	♂	306.1	31.83	10.4	306.1	37.94	12.4
6	♀	379.1	39.95	10.5	377.0	39.21	10.4
7	♀	342.0	33.81	9.9	342.0	38.57	11.3
8	♀	244.6	14.72	6.0	244.6	21.11	8.6
9	♀	298.9	28.79	9.6	298.9	27.59	9.2
10	♂	293.8	30.24	10.3	293.8	26.69	9.1
11	♂	299.6	37.11	12.4	299.5	36.80	12.3
12	♀	370.7	50.97	13.7	370.1	38.59	10.4
Average		311.7	35.83	11.4	311.5	33.93	10.9

[1] Contents of alimentary tract removed.

TABLE 18.—*Energy of body gain of rats fed vitamin-D-supplemented or vitamin-D-deficient diets as related to energy and to metabolizable energy of food*

Pair no.	Rats fed vitamin-D-supplemented diet					Rats fed vitamin-D-deficient diet					Body gain of energy of vitamin D free rats as percentage of gain of pair mate
	Food energy	Body gain		Metabolizable energy	Body gain as percentage of metabolizable energy	Food energy	Body gain		Metabolizable energy	Body gain as percentage of metabolizable energy	
		Total	As percentage of feed energy				Total	As percentage of feed energy			
	Calories	*Calories*	*Percent*	*Percent*	*Percent*	*Calories*	*Calories*	*Percent*	*Calories*	*Percent*	*Percent*
1	1,270	126.1	9.9	1,079.0	11.7	1,270	72.4	5.7	1,075.5	6.7	57.4
2	1,445	144.5	10.0	1,229.4	11.8	1,445	94.9	6.6	1,210.9	7.8	65.7
3	1,355	121.6	9.0	1,148.6	10.6	1,355	71.4	5.3	1,146.4	6.2	58.7
4	1,508	103.5	6.9	1,276.9	8.1	1,508	92.8	6.2	1,275.3	7.3	89.7
5	1,415	64.4	4.6	1,187.6	5.4	1,415	81.4	5.8	1,190.8	6.8	126.4
6	1,749	111.3	6.4	1,467.8	7.6	1,740	92.5	5.3	1,459.3	6.3	83.1
7	1,581	155.6	9.8	1,332.0	11.7	1,581	124.8	7.9	1,342.8	9.3	80.2
8	1,134	34.8	3.1	950.7	3.7	1,134	36.8	3.2	963.5	3.8	105.7
9	1,383	77.1	5.6	1,164.5	6.6	1,383	53.2	3.8	1,166.5	4.6	69.0
10	1,360	47.8	3.5	1,146.6	4.2	1,360	35.7	2.6	1,144.2	3.1	74.7
11	1,384	92.7	6.7	1,152.7	8.0	1,384	68.7	5.0	1,166.0	5.9	74.1
12	1,711	121.7	7.1	1,429.8	8.5	1,709	67.3	3.9	1,443.7	4.7	55.3
Average	1,441	100.1	6.9	1,213.8	8.2	1,440	74.3	5.1	1,215.4	6.0	74.3

TABLE 19.—*Heat loss of rats fed vitamin-D-supplemented or vitamin-D-deficient diets as related to energy of food*

Pair no.	Rats fed vitamin-D-supplemented diet			Rats fed vitamin-D-deficient diet		
	Food energy	Heat loss		Food energy	Heat loss	
		Total	As percentage of feed energy		Total	As percentage of feed energy
	Calories	*Calories*	*Percent*	*Calories*	*Calories*	*Percent*
1	1,270	952.9	75.0	1,270	1,003.1	79.0
2	1,445	1,084.9	75.1	1,445	1,116.0	77.2
3	1,355	1,027.0	75.8	1,355	1,075.0	79.3
4	1,508	1,173.4	77.8	1,508	1,182.5	78.4
5	1,415	1,123.2	79.4	1,415	1,109.4	78.4
6	1,749	1,356.5	77.6	1,740	1,366.8	78.6
7	1,581	1,176.4	74.4	1,581	1,218.0	77.0
8	1,134	915.9	80.8	1,134	926.7	81.7
9	1,383	1,087.4	78.6	1,383	1,113.3	80.5
10	1,360	1,098.8	80.8	1,360	1,108.5	81.5
11	1,384	1,060.0	76.6	1,384	1,097.3	79.3
12	1,711	1,308.1	76.5	1,709	1,376.4	80.5
Average	1,441	1,113.7	77.4	1,440	1,141.1	79.3

TABLE 20.—*Nitrogen of body gain of rats fed vitamin-D-supplemented or vitamin-D-deficient diets as related to fat and energy of body gain and to nitrogen of food*

RATS FED VITAMIN-D-SUPPLEMENTED DIET

Pair no.	Nitrogen of body gain	Fat gained		Energy gained			Nitrogen of food	
		Total	Per gram nitrogen gained	Total	As fat	As protein	Total	Utilized for body gain
	Grams	*Grams*	*Grams*	*Calories*	*Percent*	*Percent*	*Grams*	*Percent*
1	1.30	8.5	6.5	126.1	64.6	35.4	11.52	11.3
2	1.69	8.9	5.3	144.5	56.7	43.3	13.15	12.9
3	1.68	6.7	4.0	121.6	51.4	48.6	12.32	13.6
4	1.59	4.9	3.1	103.5	44.0	56.0	13.73	11.6
5	1.46	1.3	.9	64.4	18.6	81.4	12.87	11.3
6	1.70	5.6	3.3	111.3	46.5	53.5	15.98	10.6
7	1.20	11.5	9.6	155.6	70.2	29.8	14.40	8.3
8	.86	.2	.2	34.8	5.5	94.5	10.27	8.4
9	1.36	2.5	1.8	77.1	28.7	71.3	12.57	10.8
10	1.35	.5	.3	47.8	8.6	91.4	12.35	10.9
11	1.63	4.0	2.5	92.7	39.1	60.9	12.61	12.9
12	2.06	5.2	2.5	121.7	39.2	60.8	15.62	13.2
Average	1.49	5.0	3.3	100.1	39.4	60.6	13.12	11.3

RATS FED VITAMIN-D-DEFICIENT DIET

1	1.66	2.0	1.2	72.4	25.8	74.2	11.52	14.4
2	1.48	4.8	3.2	94.9	45.8	54.2	13.15	11.3
3	1.65	1.6	1.0	71.4	20.6	79.4	12.32	13.4
4	1.71	3.8	2.2	92.8	38.4	61.6	13.73	12.5
5	1.87	2.0	1.1	81.4	22.2	77.8	12.87	14.5
6	1.86	3.5	1.9	92.5	34.4	65.6	15.89	11.7
7	1.70	6.5	3.8	124.8	49.4	50.6	14.40	11.8
8	1.20	−.6	−.5	36.8	−16.0	116.0	10.27	11.7
9	1.41	1.1	.8	53.2	16.9	83.1	12.57	11.2
10	1.39	−1.0	−.7	35.7	−25.5	125.5	12.35	11.3
11	1.86	1.1	.6	68.7	13.7	86.3	12.61	14.8
12	1.91	.3	.2	67.3	4.2	95.8	15.59	12.3
Average	1.64	2.1	1.2	74.3	19.2	80.8	13.11	12.6

TABLE 21.—*Urinary carbon of rats fed vitamin-D-supplemented or vitamin-D-deficient diets as related to urinary nitrogen*

Pair no.	Rats fed vitamin-D-supplemented diet			Rats fed vitamin-D-deficient diet		
	Urinary carbon	Urinary nitrogen	Carbon nitrogen ratio	Urinary carbon	Urinary nitrogen	Carbon nitrogen ratio
	Grams	*Grams*		*Grams*	*Grams*	
1	7.14	9.03	0.8	6.88	8.62	0.8
2	7.87	9.86	.8	7.70	9.75	.8
3	7.27	9.00	.8	7.16	9.17	.8
4	8.53	9.89	.9	8.46	10.18	.8
5	8.11	9.01	.9	7.85	9.46	.8
6	9.52	11.63	.8	9.24	11.76	.8
7	8.96	11.40	.8	8.35	10.36	.8
8	6.38	8.29	.8	6.15	7.85	.8
9	7.81	9.55	.8	7.51	9.57	.8
10	7.88	8.99	.9	7.91	9.38	.8
11	8.18	9.41	.9	7.36	9.11	.8
12	9.81	11.44	.9	9.90	11.17	.9
Average	8.12	9.79	.8	7.87	9.70	.8

TABLE 22.—*Digestibility of protein and energy-producing nutriment of diets containing or lacking vitamin D, when fed to rats*

Pair no.	Rats fed vitamin-D-supplemented diet						Rats fed vitamin-D-deficient diet					
	Nitrogen			Energy			Nitrogen			Energy		
	Food	Feces	Digested	Food	Feces	Digested	Food	Feces	Digested	Food	Feces	Digested
	Grams	*Grams*	*Per cent*	*Calories*	*Calories*	*Per cent*	*Grams*	*Grams*	*Per cent*	*Calories*	*Calories*	*Per cent*
1	11.52	0.77	93.3	1,270	115.6	90.9	11.52	0.85	92.6	1,270	120.7	90.5
2	13.15	.95	92.8	1,445	132.2	90.9	13.15	1.10	91.6	1,445	149.8	89.6
3	12.32	.86	93.0	1,355	127.8	90.6	12.32	.84	93.2	1,355	128.6	90.5
4	13.73	1.02	92.6	1,508	140.2	90.7	13.73	.99	92.8	1,538	139.2	90.8
5	12.87	1.14	91.1	1,415	140.7	90.1	12.87	1.01	92.2	1,415	139.7	90.1
6	15.98	1.32	91.7	1,749	177.1	89.9	15.89	1.34	91.6	1,740	179.0	89.7
7	14.40	1.07	92.6	1,581	154.8	90.2	14.40	1.10	92.4	1,581	146.6	90.7
8	10.27	.81	92.1	1,134	113.7	90.0	10.27	.77	92.5	1,134	103.5	90.9
9	12.57	.89	92.9	1,383	133.4	90.4	12.57	.97	92.3	1,383	133.5	90.3
10	12.35	.97	92.1	1,360	130.3	90.4	12.35	.97	92.1	1,360	130.3	90.4
11	12.61	.99	92.1	1,384	145.0	89.5	12.61	.90	92.9	1,384	137.2	90.1
12	15.62	1.31	91.6	1,711	176.7	89.7	15.59	1.16	92.6	1,709	160.9	90.6
Average	13.12	1.01	92.3	1,441	140.6	90.3	13.11	1.00	92.4	1,440	139.1	90.4

PRESENTATION OF RESULTS

Both animals of a pair received the same amount of the same diet (the weight of the viosterol administered being negligible) and the animals were weighed daily in the morning before feeding. The growth curves for the entire feeding period are shown in figure 3, *A*.

The gains in gross live weight revealed 6 greater gains by the vitamin D rats, and 6 by the rats on the vitamin D deficient diet; and the gains in empty body weight showed that in 9 cases of the 12 the vitamin D rats made larger gains than did their pair mates (table 17); but the gains were small, and on neither basis of comparison were there statistically significant differences between the results of the 2 treatments.

Although the rats showed no signs of lack of vitamin A, they grew but slowly. In view of the method of food apportionment employed, the rats on the rachitogenic diet undoubtedly held back their pair mates, in food consumption and in growth. The rats on the vitamin deficient diet refused a part of their food a total of 381 times as compared with 259 refusals for the rats on the supplemented diets. This difference was statistically significant.

The development of rickets is more pronounced, on a rachitogenic diet, when growth is more rapid than it was in this experiment. However, a comparison of the X-ray photographs of the rats, taken at the end of 10 weeks, showed a distinct effect of the vitamin deficiency. Those individuals which received viosterol regularly showed normal development of the bones, while all of those on the rachitogenic diet showed malformation as shown in figure 2.

On an average, for the 12 pairs, 94.04 percent (90.21 to 96.98) of the food nitrogen was recovered in the urine, the feces, and the body gains. The nitrogen not accounted for may be considered to represent shed hair, ammonia evaporated from the urine, and analytical error.

The rats on the complete diet gained significantly more fat and energy (table 20) and eliminated more carbon in the urine (table 21) than did the rats on the vitamin-D-deficient diet.

With the vitamin-D-deficient rats there was a higher percentage utilization of nitrogen for body gain (table 20), a higher percentage of the gain was protein (table 20), and there was also a greater heat loss (table 19) than there was with the rats on the complete diet.

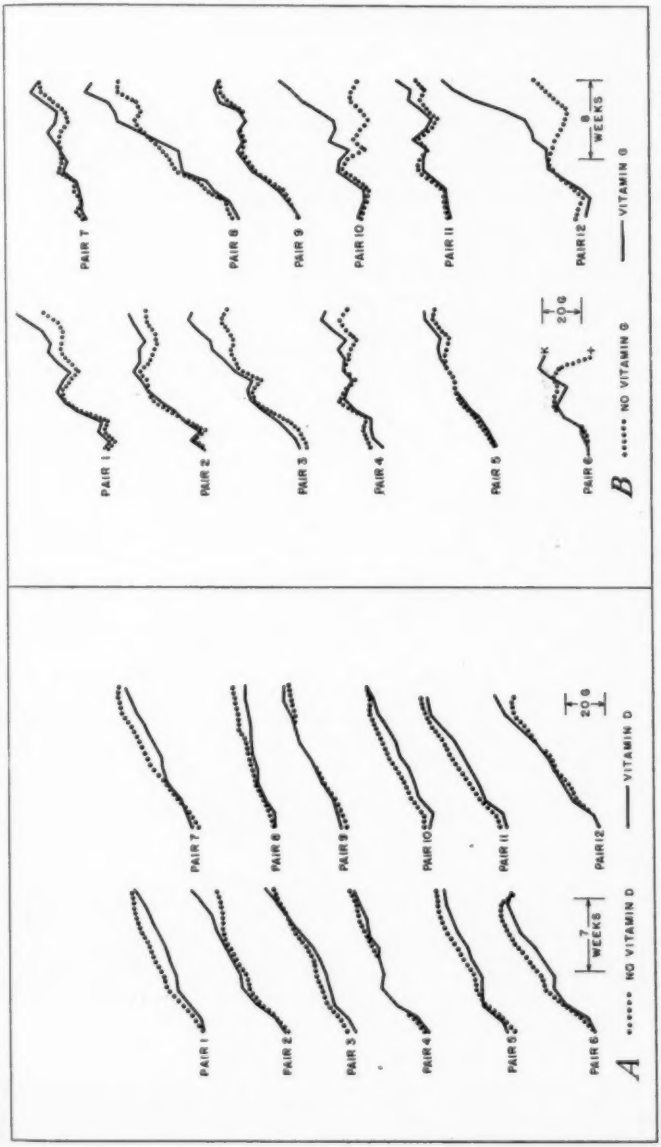

FIGURE 3.—Comparative growth of rats which received: (*A*) Equicalorie quantities of a complete diet and of a diet deficient in vitamin D, and (*B*) diets rich and poor in vitamin G.

There were no significant differences between the two groups as to the metabolizability of the energy of the rations (table 18), the digestibility of either the nitrogen or the energy of the rations (table 22), the gain in live weight (table 17), the nitrogen of the urine (table 21), the energy of the urine and feces, or the ratio of carbon to nitrogen in the urine (tables 21 and 22).

The carbon-nitrogen ratio of the urine was either 0.8 or 0.9 in every case, averaging 0.8+ with both groups of rats. The diet was high in protein (23.63 percent), and the ratio of carbon to nitrogen was about the same as in another experiment of this series in which the diet was about equally rich in protein.

THE EFFECTS OF VITAMIN G DEFICIENCY

The published work on vitamin G as affecting the utilization of food being meager and for the most part confined to observations on gain of live weight, an experiment was planned especially to determine the characteristic differences in the details of utilization of the protein and energy of food when supplied with and without adequate amounts of vitamin G. The method of experimentation was as in the foregoing studies of this series.

Kon (*17*) emphasized the necessity of determining the actual chemical composition and energy content of the gains in weight in investigations involving the relation of any indispensable nutrient to the metabolism of matter and energy; and in an earlier paper (*16*) suggested that vitamin G is intimately concerned with the general oxidative processes, though he later concluded (*17*) that its association with the metabolic processes of the body is merely comparable to that of other indispensable food ingredients.

St. Julian and Heller (*27*) found the digestibility of protein to be somewhat lower with rats which received a vitamin B_1 and a vitamin-B_2-deficient diet supplemented with vitamin B_2 alone, than when the diet was supplemented with vitamin B_1 alone, or with vitamin B_1 and B_2 together, or when no supplement was added. This finding, however, is not stressed by the authors, and they conclude that vitamins A, B_1, B_2, C, and D exert no effect on the digestibility of protein, fat, and carbohydrate.

Hassan and Drummond (*13*) noted that the heat-stable alkali-resistant fraction of the vitamin B complex in yeast extract renders very high protein diets adequate for growth.

Graham and Griffith (*11*) found a decreased utilization of food, for gain in live weight, when both vitamins B and G were supplied at a low level. They found that both vitamins were necessary for normal appetite, and that these two vitamins "seemed to depend upon the presence of each other for their activity", a finding which furnishes an example of the general interdependence of the values of food nutrients as stated by Forbes (*6*).

EXPERIMENTAL PROCEDURE

Twelve pairs of weanling rats, the pair mates being from the same litter and of the same sex, were fed, for 14 weeks, in a growth-, metabolism-, and body-analysis experiment.

The basal ration was composed as follows: Casein (vitamin-free) 18 percent, Osborne and Mendel salt mixture 4 percent, Crisco 10

percent, dextrin 64 percent, Cellu flour 2 percent, and cod-liver oil 2 percent. Vitamin B_1 was supplied in equal and sufficient amounts to both groups of rats in the form of a concentrate.[5]

Some difficulty was experienced in providing the desired difference in vitamin G content of the diet as fed to the two groups of rats. Autoclaved yeast was not suitable as a source of vitamin G because of accompanying protein and energy which it would contribute; and the use of yeast which had been exposed to ultraviolet rays seemed undesirable on account of the inconsistent results of this method of destruction of vitamin G, as reported in the literature. During the first 7 weeks of the experiment 2 methods of accomplishing the stated purpose were tried. A concentrate prepared from dried brewer's yeast having proved ineffective, an attempt was made to secure the desired difference in vitamin G by supplementing the diet for one group with untreated yeast, and for the other with a like amount of yeast previously heated, dry, at 100° C., to destroy vitamin G. Elevehjem and his associates (*4*), working with chicks, secured about 50 percent destruction of vitamin G by heating baker's yeast at 100° for 144 hours. In the light of this finding, samples of yeast were heated in a Freas oven at 100° for 14 and 29 days, respectively; but neither the growth (fig. 3, *B*) nor the appearance of the rats indicated vitamin G deficiency in the ration supplemented with the heated yeast. Block and Farquhar (*2*) have recently published a similar finding.

The supplement of dry heated yeast was, therefore, discontinued at the end of the seventh week, and an aqueous extract of Eli Lilly & Co.'s liver concentrate no. 343 was prepared according to the method of Guha (*12*). That liver contains an abundance of vitamin G has been quite well established, and there is evidence that it may also contain other factors especially favorable to growth and reproduction. It is recognized, therefore, that the water extract of the liver concentrate used in this experiment may have furnished more than a single factor in exerting its influence on the utilization of the protein and energy of the food.

Beginning with the eighth week (fig. 3, *B*) one rat of each pair received daily 0.5 cc of the extract, the high potency of which permitted its use in small amounts, thus minimizing the difference between the protein and energy intake of the two groups of rats. All supplements fed were carefully weighed and analyzed, and were considered in the computation of the intake of protein and energy. The rats given the supplemented diet received (on an average) 18 Calories (1.07 percent) more feed energy and 0.37 g (4 percent) more nitrogen than did their pair mates, in the course of the 14 weeks' experiment.

The rat of pair no. 6, which received the vitamin-G-deficient diet, died at the end of the tenth week, as a result of vitamin G avitaminosis, indicating that the dry-heating of the yeast used earlier in the experiment was not entirely ineffective in destroying vitamin G. The pair mate of this rat was killed for purposes of comparison.

In order to prevent more of the vitamin-G-deficient rats from dying, and to permit limited growth, small weighed amounts of yeast were added to the vitamin-G-deficient diet from time to time: observations on the condition and appearance of the rats were also made every few days and rectal temperatures of the rats were taken daily during the last 2 weeks of the experiment.

[5] This concentrate was kindly donated by Parke, Davis & Co.

Various degrees of success have been reported by workers who have attempted to produce vitamin-G-deficiency dermatitis. At the end of this experiment there were 7 cases (out of a possible 12) of vitamin G avitaminosis, as indicated either by outward appearance or by autopsy, with individual variations as to symptoms and as to severity.

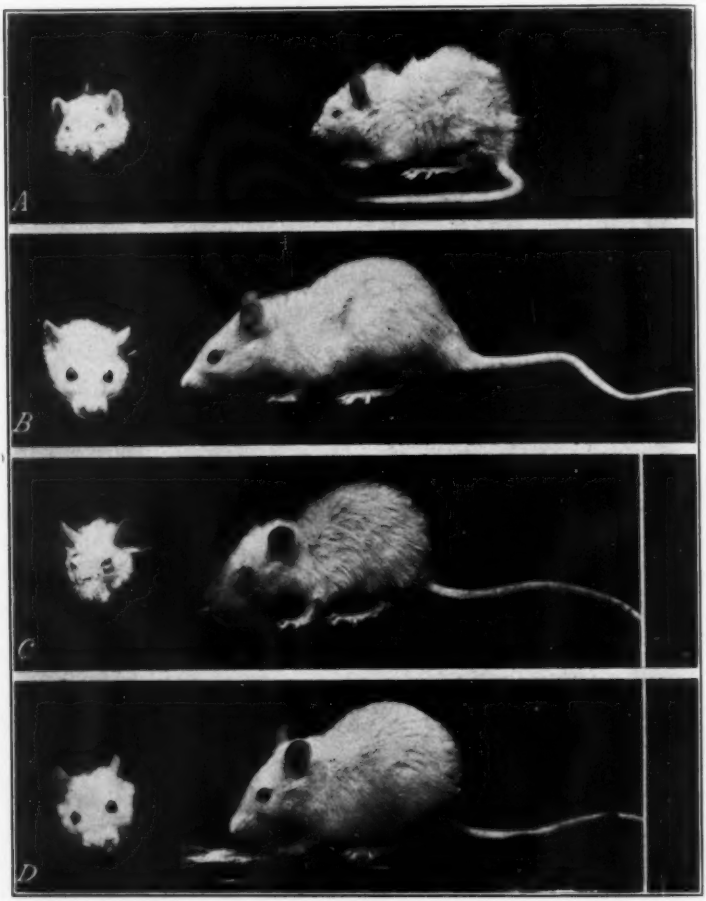

FIGURE 4.—Rats of pair nos. 10 and 12, respectively, *A* and *C* being vitamin G deficient, and *B* and *D* being normal rats.

The deficient rats of pairs 3, 4, 7, and 11 developed mild pellagralike conditions, while the symptoms of the deficient rats of pairs 6, 10, and 12 resembled closely those reported by Goldberger and Lillie (*9*) and by Chick and Roscoe (*3*). The appearance of pairs 10 and 12 near the end of the experiment, is shown in figure 4.

Since coprophagy was encountered in a few cases, a wire tunnel was devised (fig. 5) which, when attached to the feed cup, prevented the rat from turning round during the taking of feed. While this device by no means wholly prevented the eating of the feces, it aided materially, as judged by the decrease in number of small particles of feces found in the crystallizing dish below the cage.

At the end of the experiment the rats were killed, and the contents of the alimentary tract were discarded as in the other experiments of this series. Observations made on the organs of the rats at this time revealed, in some cases, no difference between pair mates, but in pairs 3, 6, 7, 10, and 12 enteritis and accumulated intestinal gas were manifest, especially in the small intestine, these conditions being most marked in pairs 6, 10, and 12. All of the rats which received the liver supplement appeared healthy and normal throughout the experiment and at autopsy.

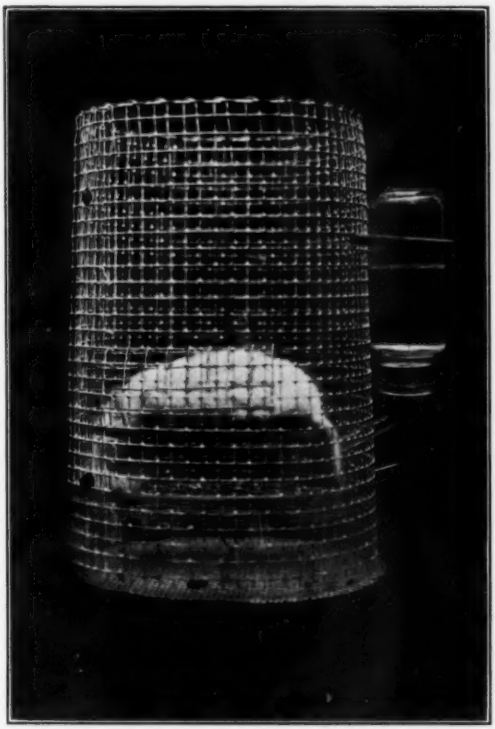

FIGURE 5.—A rat eating inside a screen cylinder which diminished coprophagy.

PRESENTATION OF RESULTS

A statistical study of the weekly gains in live weight revealed a superiority of performance by the rats which received the supplemented diet, the significance of which is indicated by odds of 1,000 to 1; also there were 516 refusals of feed, 356 of which were from the rats on the deficient diet. That the rats which received the vitamin G deficient diet limited the food consumption of their pair mates is, therefore, indicated by overwhelming odds. This fact, however, is not necessarily an indication that vitamin G has a direct, specific effect on appetite, but may signify only that the lesser food consumption is incidental to vitamin G avitaminosis.

Except for pair 6, the data in tables 23 to 28 represent an experimental period of 14 weeks. Early in the experiment there were no symptoms of vitamin G avitaminosis, probably because of a small amount of vitamin G in the deficient ration, and because of a body store of vitamin G. Well-defined results obtained from the whole period of 14 weeks, therefore, may be interpreted with confidence. Differ-

ences in average values for the two groups of rats presented in the tables should be considered to have directional rather than quantitative significance, however, since the values obtained depend on the extent of the concurrent avitaminosis. In general, the greatest differences between pair mates shown in the tables correspond to the greatest differences between pair mates as judged by their behavior and general appearance—toward the end of the experiment and at autopsy.

TABLE 23.—*Gain in body weight* [1] *of rats fed vitamin-G-supplemented or vitamin-G-deficient diets as related to dry matter of food*

Pair no.	Sex	Rats fed vitamin-G-supplemented diet			Rats fed vitamin-G-deficient diet		
		Food eaten (dry matter)	Gain in body weight	Ratio of gain to dry matter of food	Food eaten (dry matter)	Gain in body weight	Ratio of gain to dry matter of food
		Grams	*Grams*	*Percent*	*Grams*	*Grams*	*Percent*
1	♂	312.4	44.97	14.4	307.4	30.38	9.9
2	♀	346.8	38.07	11.0	342.0	28.84	8.4
3	♂	361.6	52.82	14.6	356.6	39.12	11.0
4	♀	318.8	28.76	9.0	313.9	14.18	4.5
5	♀	304.0	34.03	11.2	299.3	30.64	10.2
6	♂	230.0	24.85	10.8	226.5	5.00	2.2
7	♂	317.2	25.92	8.2	313.2	25.38	8.1
8	♂	452.6	75.61	16.7	447.9	59.05	13.2
9	♀	385.9	38.33	9.9	381.7	40.99	10.7
10	♀	333.4	38.92	11.7	329.9	1.61	.5
11	♀	338.8	26.57	7.8	335.0	16.76	5.0
12	♂	403.4	67.23	16.7	398.5	19.30	4.8
Average		342.1	41.34	11.8	337.7	25.94	7.4

[1] Contents of alimentary tract removed.

TABLE 24.—*Energy of body gain of rats fed vitamin-G-supplemented or vitamin-G-deficient diets as related to energy, and to metabolizable energy, of food*

Pair no.	Rats fed vitamin-G-supplemented diet					Rats fed vitamin-G-deficient diet					Body gain of energy of vitamin G deficient rats as percentage of pair mate
	Food energy	Body gain		Metabolizable energy	Body gain as percentage of metabolizable energy	Food energy	Body gain		Metabolizable energy	Body gain as percentage of metabolizable energy	
		Total	As percentage of food energy				Total	As percentage of food energy			
	Calories	*Calories*	*Per cent*	*Calories*	*Percent*	*Calories*	*Calories*	*Per cent*	*Calories*	*Percent*	*Percent*
1	1,553.3	91.2	5.9	1,432.5	6.4	1,532.1	45.4	3.0	1,415.4	3.2	49.8
2	1,724.8	106.0	6.1	1,581.1	6.7	1,705.4	74.2	4.4	1,553.0	4.8	70.0
3	1,798.8	96.4	5.4	1,656.1	5.8	1,778.5	59.9	3.4	1,641.1	3.6	62.1
4	1,584.8	70.6	4.5	1,447.2	4.9	1,564.9	31.1	2.0	1,430.9	2.2	44.1
5	1,510.8	61.3	4.1	1,387.6	4.4	1,491.8	65.1	4.4	1,341.6	4.9	106.2
6	1,143.4	38.7	3.4	1,056.0	3.7	1,129.2	−7.5	.....	1,040.0	.....	.....
7	1,576.7	30.7	1.9	1,440.2	2.1	1,560.5	46.2	3.0	1,443.8	3.2	150.5
8	2,253.5	159.0	7.1	2,080.6	7.6	2,234.3	91.5	4.1	2,076.0	4.4	57.5
9	1,920.5	73.9	3.8	1,759.8	4.2	1,903.5	75.5	4.0	1,723.7	4.4	102.2
10	1,658.7	87.0	5.2	1,513.4	5.7	1,644.9	−6.5	.....	1,436.9	.....	.....
11	1,684.7	59.8	3.5	1,549.4	3.9	1,669.3	31.2	1.9	1,515.0	2.1	52.2
12	2,007.7	153.1	7.6	1,858.9	8.2	1,987.0	33.0	1.7	1,819.1	1.8	21.6
Average	1,701.5	85.6	4.9	1,563.6	5.3	1,683.5	44.9	.....	1,536.4	.....	.....

TABLE 25.—*Heat loss of rats fed vitamin-G-supplemented or vitamin-G-deficient diets as related to energy of food*

Pair no.	Rats fed vitamin-G-supplemented diet			Rats fed vitamin-G-deficient diet		
	Food energy	Heat loss		Food energy	Heat loss	
		Total	As percentage of food energy		Total	As percentage of food energy
	Calories	*Calories*	*Percent*	*Calories*	*Calories*	*Percent*
1	1,553.3	1,341.3	86.4	1,532.1	1,370.0	89.4
2	1,724.8	1,475.1	85.5	1,705.4	1,478.8	86.7
3	1,798.8	1,559.7	86.7	1,778.5	1,581.2	88.9
4	1,584.8	1,376.6	86.9	1,564.9	1,399.8	89.4
5	1,510.8	1,326.3	87.8	1,491.8	1,276.5	85.6
6	1,143.4	1,017.3	89.0	1,129.2	1,047.5	92.8
7	1,576.7	1,409.5	89.4	1,560.5	1,397.6	89.6
8	2,253.5	1,921.6	85.3	2,234.3	1,984.5	88.8
9	1,920.5	1,685.9	87.8	1,903.5	1,648.2	86.6
10	1,658.7	1,426.4	86.0	1,644.9	1,443.4	87.8
11	1,684.7	1,489.6	88.4	1,669.3	1,483.8	88.9
12	2,007.7	1,705.8	85.0	1,987.0	1,786.1	89.9
Average	1,701.5	1,477.9	87.0	1,683.5	1,491.5	88.7

TABLE 26.—*Nitrogen of body gain of rats fed vitamin-G-supplemented or vitamin-G-deficient diets as related to fat and energy of body gain and to nitrogen of food*

RATS FED VITAMIN-G-SUPPLEMENTED DIET

Pair no.	Nitrogen of body gain	Fat gained		Energy gained			Nitrogen of food	
		Total	Per gram of nitrogen gained	Total	As fat	As protein	Total	Utilized for body gain
	Grams	*Grams*	*Grams*	*Calories*	*Calories*	*Calories*	*Grams*	*Percent*
1	1.87	3.0	1.6	91.2	27.0	64.2	8.81	21.2
2	1.60	5.8	3.6	106.0	53.2	52.8	9.74	16.4
3	2.23	2.7	1.2	96.4	24.7	71.7	10.14	22.0
4	1.40	2.8	2.0	70.6	25.7	44.9	8.96	15.6
5	1.63	1.0	.6	61.3	9.1	52.2	8.60	19.0
6	1.23	0.0	0.0	38.7	0.0	38.7	6.47	19.0
7	1.18	−.8	.....	30.7	−7.3	38.0	8.93	13.2
8	2.80	7.2	2.6	159.0	66.7	92.3	12.51	22.4
9	1.56	2.4	1.5	73.9	23.0	50.9	10.74	14.5
10	1.54	3.9	2.5	87.0	36.6	50.4	9.37	16.4
11	1.21	2.2	1.8	59.8	21.1	38.7	9.51	12.7
12	2.68	7.0	2.6	153.1	65.8	87.3	11.23	23.9
Average	1.74	3.1	.....	85.6	28.8	56.8	9.58	18.0

RATS FED VITAMIN-G-DEFICIENT DIET

1	1.48	−0.2	.....	45.4	−2.4	47.8	8.43	17.6
2	1.27	3.5	2.8	74.2	31.4	42.8	9.32	13.6
3	1.76	.4	.2	59.9	3.0	56.9	9.70	18.1
4	.98	.3	.3	31.1	2.1	29.0	8.57	11.4
5	1.34	2.2	1.6	65.1	19.5	45.6	8.19	16.4
6	.49	−2.3	.....	−7.5	−20.5	13.0	6.17	7.9
7	1.14	.9	.8	46.2	8.4	37.8	8.58	13.3
8	2.38	1.4	.6	91.5	13.2	78.3	12.10	19.7
9	1.61	2.5	1.6	75.5	23.8	51.7	10.37	15.5
10	.30	−1.7	.....	−6.5	−15.7	9.2	9.07	3.3
11	.92	.1	.1	31.2	1.3	29.9	9.19	10.0
12	1.01	0.0	0.0	33.0	1.0	32.0	10.88	9.3
Average	1.22	0.6	.....	44.9	5.4	39.5	9.21	13.0

TABLE 27.—*Urinary carbon of rats fed vitamin-G-supplemented and vitamin-G-deficient diets as related to urinary nitrogen*

Pair no.	Rats fed vitamin-G-supplemented diet			Rats fed vitamin-G-deficient diet		
	Urinary carbon	Urinary nitrogen	Carbon-nitrogen ratio	Urinary carbon	Urinary nitrogen	Carbon-nitrogen ratio
	Grams	*Grams*		*Grams*	*Grams*	
1	4.27	6.29	0.68	5.00	6.28	0.80
2	4.66	7.58	.61	5.09	7.03	.72
3	3.93	7.01	.56	4.89	6.89	.71
4	4.74	6.93	.68	5.56	6.92	.80
5	4.05	6.45	.63	4.62	6.36	.73
6	2.41	4.79	.50	2.92	5.07	.58
7	4.40	6.66	.65	4.61	6.37	.72
8	5.19	8.65	.60	5.33	8.72	.61
9	5.16	8.17	.63	4.82	7.69	.63
10	4.39	6.99	.63	10.94	6.92	1.59
11	4.54	7.41	.61	5.76	7.35	.78
12	4.86	7.68	.63	5.92	8.48	.70
Average	4.38	7.06	.62	5.46	7.01	.78

TABLE 28.—*Digestibility of nitrogen and energy-producing nutriment of diets containing or lacking vitamin G, when fed to rats*

RATS FED VITAMIN-G-SUPPLEMENTED DIET

Pair no.	Nitrogen			Energy		
	Food	Feces	Digested	Food	Feces	Digested
	Grams	*Grams*	*Percent*	*Calories*	*Calories*	*Percent*
1	8.81	0.56	93.6	1,553.3	71.8	95.4
2	9.74	.62	93.6	1,724.8	89.8	94.8
3	10.14	.76	92.5	1,798.8	95.4	94.7
4	8.96	.67	92.5	1,584.8	83.5	94.7
5	8.60	.59	93.1	1,510.8	75.5	95.0
6	6.47	.55	91.5	1,143.4	57.3	95.0
7	8.93	.70	92.2	1,576.7	85.0	94.6
8	12.51	.88	93.0	2,253.5	111.5	95.1
9	10.74	.69	93.6	1,920.5	100.1	94.8
10	9.37	.81	91.4	1,658.7	94.0	94.3
11	9.51	.75	92.1	1,684.7	82.1	95.1
12	11.23	.72	93.6	2,007.7	91.8	95.4
Average	9.58	.69	92.7	1,701.5	86.5	94.9

RATS FED VITAMIN-G-DEFICIENT DIET

1	8.43	0.46	94.5	1,532.1	59.8	96.1
2	9.32	.64	93.1	1,705.4	94.5	94.5
3	9.70	.56	94.2	1,778.5	81.4	95.4
4	8.57	.57	93.3	1,564.9	71.9	95.4
5	8.19	.63	92.3	1,491.8	97.5	93.5
6	6.17	.50	91.9	1,129.2	53.6	95.3
7	8.58	.53	93.8	1,560.5	64.0	95.9
8	12.10	.71	94.1	2,234.3	95.4	95.7
9	10.37	.84	91.9	1,903.5	122.6	93.6
10	9.07	.88	90.3	1,644.9	94.8	94.2
11	9.19	.79	91.4	1,669.3	89.0	94.7
12	10.88	.90	91.7	1,987.0	98.6	95.0
Average	9.21	.67	92.7	1,683.5	85.3	94.9

The rats which received the supplemented diet averaged 0.29° C. higher in body temperature than did their pair mates, but statistical treatment of the data reveals odds of only 4 to 1 that this difference did not occur by chance. It may be pointed out, however, that this

finding does not contra-indicate a lower level of general oxidative metabolism, inasmuch as an abnormal body temperature signifies a disturbance of the heat-regulatory mechanism rather than a change in heat production. Findlay (*5*) has reported a drop in body temperature as a terminal feature of vitamin G avitaminosis.

The superiority of the gains in body weight of the rats which received the vitamin-G-supplemented diet is shown in table 23, the odds being about 525 to 1 that a given amount of the complete diet produced more gain in body weight than did a like amount of a similar diet which was deficient in vitamin G. No marked differences in live weight developed until the supplement of liver extract was added (fig. 1, *D*).

The body gain in energy (table 24) was, on an average, significantly greater in the case of the rats which received the vitamin-G-supplemented diet, though in three cases (pairs 5, 7, and 9) the difference appeared to be in favor of the deficient diet. The deficient rats of these three pairs, throughout the experiment, exhibited little evidence of avitaminosis. Conversely, the bodies of two of the rats (from pairs 6 and 10) on the deficient diet contained less energy at the end of the experiment than at the beginning; and these two pairs exhibited the greatest differences in appearance between pair mates, both before death and at autopsy.

The deficient rat of pair 6, which died at the end of the tenth week, showed lesions on the paws and nose, a biometrical symmetry in loss of hair, a scaliness of the skin where the hair had fallen out, and dermatitis over the entire body. At autopsy there were noted enteritis, especially of the ileum, gas in stomach and intestine, and mucuous colitis in the small intestine. The mate of this rat, killed when the deficient rat died, appeared to be normal.

The appearance of the rats of pairs 10 and 12, at about the end of the experiment, is shown in figure 4. It is to be noted (table 24) that the rats of these pairs show marked differences in the body gain of energy. The significance of the greater storage of energy by the rats which received the vitamin-G-supplemented diet, on the basis of the data in table 24, is indicated by odds of about 400 to 1.

The heat loss (table 25) was somewhat higher with the deficient rats than with the pair mates, the significance of the difference being expressed by odds of 7 to 1.

The division of the gain of energy into fat and protein (table 26) is based on the actual energy as determined in the ether extract and on the nitrogen of the extraction residue of the rat bodies at the end of the experiment, both of these values being corrected for the amount of fat and protein in the rats at the start of the experiment, as determined by the analysis of a control group of 10 similar rats.

It is of interest to note that the calories of energy gained, as measured in the body extraction residue, compare favorably with a computed value obtained by multiplying the grams of protein ($N \times 6.00$) gained by the factor 5.7. In the case of the rat which died during the experiment there is a considerable discrepancy, but with the remaining 23 rats the computed value compares well enough with the observed value (average, 105.7 percent) to justify the designation of fat and protein to the ether extract and the extraction residue, respectively.

On an average, 66.4 percent of the total energy stored by the vitamin-G-supplemented rats was in the form of protein, whereas

88.0 percent of the total stored by their pair mates was in this form. Thus, though the total gain of energy and the gain of energy as protein are considerably greater with the rats which received the supplemented diet, their pair mates were relatively more restricted in their ability to store fat than they were to store protein. Whether this fact is a characteristic effect of vitamin G deficiency, or whether it merely represents the strong inherent tendency of the young animal to synthesize protein, is not indicated.

There was no significant difference in the digestibility of the protein and energy of the two rations (table 28).

The carbon-nitrogen ratios in the urine are given in table 27. The significance of the higher ratios obtained from the deficient rats is indicated by odds of 35 to 1. These results agree with those of Kon (*16*), who obtained an increased carbon-nitrogen ratio in the urine of rats kept on a diet deficient in vitamin G. The higher carbon-nitrogen ratios result from a higher amount of carbon in the urine, the nitrogen content of urine from the two groups being almost identical. Lack of vitamin G seems to prevent the normal oxidation of carbon compounds, which then appear, in increased quantities, in the urine.

The energy of the urine of the rats which received the supplemented diet averaged 51.4 calories, as compared with 61.8 calories for their pair mates, the significance of the difference being expressed by odds of 32 to 1.

SUMMARY

Two metabolism- and body-analysis experiments, of about 90 and 107 days' duration, respectively, were conducted with 32 rats, fed by the paired method, for the comparison of white and yellow corn, as components of approximately complete diets, as affecting the utilization of nitrogen- and energy-producing nutrients.

The rats which received white corn in the first experiment manifested xerophthalmia after 90 days' feeding; but the rats which received white corn in the second experiment did not show this disorder.

In the first experiment the yellow-corn ration was superior to the white in respect to (1) palatability, (2) gain in weight, (3) gain in energy, and (4) proportion of the energy gained which was present as fat. On the other hand, the white-corn ration was superior to the yellow in (1) digestibility of food nitrogen and (2) digestibility of food energy.

In the second experiment the yellow-corn diet was shown to be definitely superior to the white corn in (1) palatability, (2) gain in live weight, (3) grams of nitrogen gained, (4) percentage of the energy gained as protein, and (5) percentage of food nitrogen stored. The greater gain in live weight of the rats fed yellow corn consisted of protein and water. The rats fed white corn exceeded the rats fed yellow corn, however, in (1) digestibility of the food energy, (2) percentage of food energy gained as fat, (3) fat gained per gram of nitrogen gained, (4) elimination of carbon in the urine, and (5) elimination of nitrogen in the urine.

Considering the two experiments together, the yellow-corn ration definitely excelled the white-corn ration only in palatability and in gain produced in body weight; while the white-corn ration excelled in digestibility of food energy. In other respects the two experiments,

so far as they covered the same observations, were not in definitely significant agreement.

While yellow corn is normally more nutritious than white corn and normally contains much more vitamin A, and while the particular lots of yellow and of white-corn used in these experiments differed as to nutritive value, it is considered obvious, in the light of these results and of common knowledge of the composition of corn, that the difference in color of white and yellow corn is not positively indicative of the usual differences in nutritive value.

The effects of vitamin A deficiency on the utilization of energy-producing nutriment and protein were studied in a growth-, metabolism-, and body-analysis experiment with 24 weanling albino rats, which were fed, by the paired feeding method, for 84 days, in a comparison of a complete diet with a similar one which differed only in being deficient in vitamin A—the caloric intake being the same with both groups.

The vitamin-A-deficient rats were maintained in only moderate A depletion, and the experiment was terminated soon after avitaminosis had become acute and general among the deficient group.

The complete diet exceeded the vitamin-A-deficient diet only in palatability and in gain in live weight and in nitrogen.

The rats on the vitamin-A-deficient diet eliminated slightly more carbon and energy in the urine than did the rats on the complete diet, but there were no statistically significant differences between the performance of the two groups with respect to digestibility or metabolizability of the diets, nitrogen in the urine, heat production, gain of fat or of energy, or gain of fat per gram of nitrogen gained.

In its early stages the most prominent effect of vitamin A deficiency was to depress the appetite.

The effects of vitamin D deficiency on the utilization of energy-producing nutriment and protein were studied, with the albino rat, by the paired feeding method, in a growth-, metabolism-, and body-analysis experiment of 10 weeks' duration.

The differences in the response of the rats to the complete and the vitamin-D-deficient diets were compared by statistical analysis.

With equicaloric intake by both groups, the rats on the vitamin-D-deficient diet exceeded their pair mates on the complete diet in utilization of the food nitrogen for body increase, in the percentage of the body gain which was protein, and in heat loss.

The rats on the complete diet exceeded their pair mates on the vitamin-D-deficient diet in appetite, gain of fat and energy, and elimination of carbon in the urine.

There were no significant differences between the two groups of rats in the metabolizability of the energy of the diets, the digestibility of either the nitrogen or the energy of the diets, the gain in live weight, the nitrogen of the urine, the energy of the urine and the feces, or the ratio of carbon to nitrogen in the urine.

The diets contained 23.63 percent protein; and the ratio of carbon to nitrogen, in the urine, with the rats on the vitamin-D-deficient as well as those on the complete diet, was 0.8+.

Twelve pairs of young growing rats were fed by the paired feeding method for 14 weeks, with quantitative collection of feces and urine, and analysis of the rat bodies, to determine the effects of vitamin G deficiency on the utilization of the food protein and energy.

A water extract of a liver concentrate proved to be a satisfactory source of vitamin G, and a small amount of this extract was given daily to one rat of each pair.

The deficiency of vitamin G depressed the appetite, the growth, the synthesis of protein and of fat, and the storage of energy.

The deficiency of vitamin G did not appreciably affect the digestibilty of the protein and energy of the diet, nor did it exert any certain effect on the heat production.

The deficiency of vitamin G had the effect of increasing the carbon-nitrogen ratio in the urine, thus indicating a relationship between the oxidative processes and this vitamin.

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ERGOT AS A FACTOR IN THE NUTRITIVE VALUE OF RYE FOR RATS AND SWINE [1]

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INTRODUCTION

In view of the numerous reports (*4*, *6*, *7*, *8*, *9*)[2] of cases of ergotism both in livestock and human beings, and especially in view of the fact that rye is but rarely free from ergot (*Claviceps purpurea* (Fr.) Tul.), it is surprising that its effect upon the nutritive value of rye has never been thoroughly investigated. So far as the writers are aware, the only investigation of this nature which has any significance from the standpoint of livestock feeding is that of Sackville and Sinclair.[3] These authors reported that rye containing 1.46 percent of ergot was worth only 92 percent as much as pure rye, for the former was so distasteful that it was impossible to get the pigs to eat sufficient quantities of it to make satisfactory gains.

In a previous investigation (*3*), it was found that when the ergot was removed from rye the rye was eaten readily, even when not mixed with protein supplements. Furthermore, there was never any indication of the disturbances which so commonly occur when rye is fed to swine. The possibility that such disturbances might be due to the small amounts of ergot which are usually present in rye was investigated with both rats and swine. The results of the investigation are presented in this paper. Since a review of the literature has already been given (*3*), it is omitted here.

EXPERIMENTS WITH RATS

EFFECT OF 1 PERCENT OF ERGOT UPON GROWTH

It was desired to determine first whether ergot has any effect upon growth when the food intake of ergotized diets and of basal diets are equalized for pairs of rats of the same age, weight, and sex. Accordingly, ergot obtained from a drug company was incorporated at a 1 percent level in a diet containing rye and at the same level in a purified diet. The 1 percent of ergot replaced 1 percent of dextrin in each basal diet. The basal diet in which rye was used consisted of 91.74 percent of ergot-free rye, 4.76 percent lard, 2.50 percent salt mixture, and 1 percent dextrin. The purified diet consisted of 66 percent dextrin, 20 percent casein, 10 percent lard, and 4 percent salt mixture. Six pairs of male rats were fed the rye diets and 3 pairs of females and 1 pair of males were fed the purified diets. Each rat used in these experiments, as well as in the experiments that followed, was given daily in addition to the basal diet a supplement of 500 mg of yeast tablets

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[2] Reference is made by number (italic) to Literature Cited, p. 45.

[3] SACKVILLE, J. P., and SINCLAIR, R. D. BARLEY VS. RYE FOR FATTENING PIGS. Alberta Univ. Dept. Anim. Husbandry Mimeographed Rept. Expt. 49. 1923.

which had been immersed in cod-liver oil. In all diets the salt mixture used was that of Hawk and Oser (*2*).

The composite growth curves of the rats of the first two experiments are shown in figure 1, *A* to *D*. The lack of growth of the rats receiving the rye diets and the slight growth of those receiving the purified diets was occasioned by low food intake. The former consumed 17.5 g and the latter 25.06 g of food per rat per week during 4 weeks.

Although the addition of ergot apparently rendered the rye diet more unpalatable than the purified diet, a similar relationship has

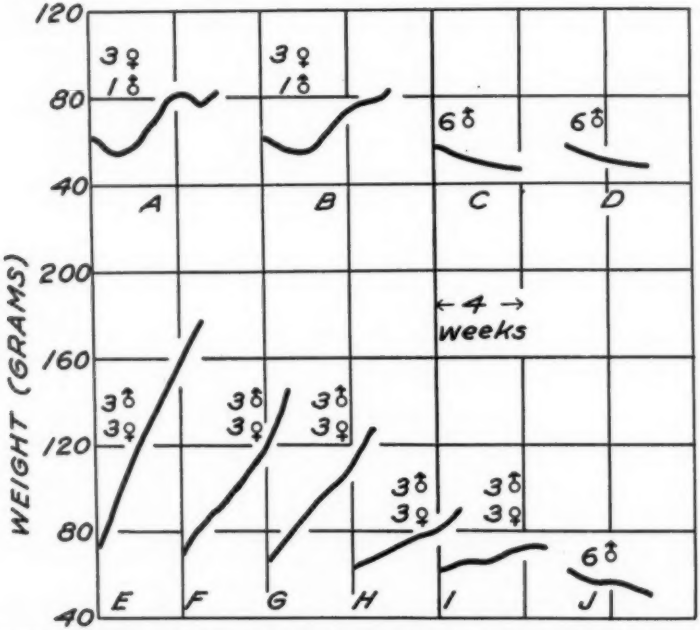

FIGURE 1.—Average growth of groups of rats fed different rations. *A*, Purified basal ration; *B*, purified basal ration plus 1 percent ergot; *C*, rye basal ration; *D*, rye basal ration plus 1 percent ergot; *E*, rye basal ration plus liver meal; *F*, rye basal ration; *G*, rye basal ration plus liver meal plus 0.1 percent ergot; *H*, rye basal ration plus liver meal plus 0.5 percent ergot; *I*, rye basal ration plus liver meal plus 1 percent ergot; *J*, rye basal ration plus 1 percent ergot.

been found between the consumption of a diet containing 90 percent or more of ergot-free rye and the consumption of a purified diet when both were offered ad libitum. This difference in food consumption may not have been entirely a matter of palatability but may have been due in part to the higher protein content of the purified diet.

Four of the rats that received the rye diet containing ergot and two of the controls had died of starvation by the end of 6 weeks. There was no mortality among the rats that received the ergot as a part of the purified diet. Growth of the controls in each experiment was but little better than that of their pair mates because of their restricted food allowance. There were no outward manifestations of ergotism in any of these rats.

EFFECT OF LIVER MEAL IN OVERCOMING UNPALATABILITY OF DIETS CONTAINING ERGOT AT 1-, 0.5-, AND 0.1-PERCENT LEVELS

Because of the pronounced effect of 1 percent of ergot upon food intake an effort was made to determine the effect upon food consumption of lower percentages of ergot and to find whether a liver meal (a special product made from livers) which had previously been shown to enhance greatly the palatability of the pruified diet and of diets containing rye (*3*), would overcome the distastefulness of ergot-treated rations.

The basal diet consisted of the following: Rye, 90 percent; dextrin, 7.32 percent; and salt mixture, 2.68 percent. Five modifications of this diet were fed: (1) with 1 percent of ergot; (2) with 6.32 percent of liver meal; and (3–5) with 6.32 percent of liver meal and 1.0, 0.5, and 0.1 percent, respectively, of ergot. The ergot or liver meal and ergot replaced an equivalent weight of dextrin in each diet. After the second week the liver meal in the diet containing 6.32 percent liver meal and 1 percent ergot was increased to 20 percent (fig. 1, *G*). Six rats, 3 males and 3 females, were fed each diet, except the first modification, which was fed to 6 males. All diets were consumed ad libitum.

The composite growth curves for the rats in this series of experiments are given in figure 1, *E* to *J*. An inspection of these curves reveals that the liver meal, even when comprising 20 percent of the diet, did not overcome the distaste of the rats for the diets containing ergot and that ergot retarded growth even when fed as 0.1 percent of the diet. The addition of the liver meal to the rye basal diet caused a pronounced increase in the food consumption and growth of the rats, as shown in table 1.

TABLE 1.—*Average weekly quantity (grams) of food consumed by rats in a comparison of the effect of ergot when fed with rye*

Ration and sex of rats in groups	Food consumed for week indicated				
	1	2	3	4	5
Basal (3 male, 3 female)	58.6	58.6	68.3	72.1	75.8
Basal + 1.0 percent ergot (6 male)	26.1	21.8	22.2	19.6	.....
Basal + liver meal (3 male, 3 female)	80.1	107.3	96.6	91.3	109.5
Basal + liver meal + 1.0 percent ergot (3 male, 3 female)	31.1	28.6	52.0	25.4	18.7
Basal + liver meal + 0.5 percent ergot (3 male, 3 female)	46.8	37.2	51.8	41.2	54.6
Basal + liver meal + 0.1 percent ergot (3 male, 3 female)	65.8	75.8	71.8	61.6	79.8

GROWTH OF RATS RECEIVING CORN AND RYE DIETS WITH AND WITHOUT ERGOT

An effort was made to find whether a diet made up of ergot and corn would be as unpalatable as a diet of ergot and rye.

The rye diet contained 82.17 percent rye; 12.47 percent "trinity" (2 parts tankage, 1 part linseed meal, and 1 part alfalfa meal); 1.97 percent salt mixture; 0.53 percent agar; 1.96 percent lard; and 1.0 percent dextrin. The corn diet contained 79.27 percent corn; 12.47 percent trinity; 1.97 percent salt mixture; 0.98 percent agar; 1.08 percent lard; and 4.23 percent dextrin. These diets were isodynamic. They were fed ad libitum and also in a paired feeding test. Two other groups of rats were fed each of the basal diets in which 0.5 and 1 percent, respectively, of ergot replaced an equivalent weight of dextrin. This ergot was not from the same supply as the ergot used in the experiments reported in sections 1 and 2 but was purchased from the

same company. Three male and three female rats were fed each diet. The growth and food consumption data for this series of experiments are tabulated in table 2.

TABLE 2.—*Average weekly quantity (grams) of food consumed and gain in weight by rats for 4-, 8-, 10-, and 12-week periods in the comparison of the effect of ergot in diets of corn and rye*

Ration	4 weeks		8 weeks		10 weeks		12 weeks	
	Food	Gain	Food	Gain	Food	Gain	Food	Gain
Corn diet+1.0 percent ergot	50.5	11.0	59.7	13.6	61.9	12.6	63.4	11.5
Rye diet+1.0 percent ergot	54.0	12.7	58.0	12.0	60.4	11.4	61.0	9.9
Corn diet+0.5 percent ergot	60.4	15.0	70.7	16.0	73.1	14.5	75.0	13.3
Rye diet+0.5 percent ergot	52.6	13.5	61.3	12.7	63.9	11.4	66.4	10.6
Corn basal diet (ad libitum)	72.7	19.7	82.6	18.5	84.4	16.6	85.5	15.0
Rye basal diet (ad libitum)	73.0	18.5	81.8	17.0	83.0	14.9	85.9	14.4
Corn basal diet (paired)	63.6	14.3	74.0	14.8	75.1	13.4	.....	.....
Rye basal diet (paired)	63.6	15.2	74.0	14.5	75.1	13.3	.....	.....

The fact that all the rats on the rye diets containing 1.0 percent of ergot grew fairly well was surprising in view of the negligible growth and high mortality of the rats on the rye plus liver diets containing 1.0 percent of the ergot used in the first ergot experiments. There were no outward manifestations in any of the animals which could be considered as due to the ergot. There must indeed be great differences between different samples of ergot in the amount of the principles present which account for its distastefulness. Apparently this has been recognized by livestock feeders, for McNeil and Pammel (*5*), referring to ergotized hay, state: "Such hay is not always dangerous even when infested with ergot * * * ." There is but little indication that the ergot was more detrimental when fed with rye than when fed with corn. That there was nothing in the rye used in this experiment which retarded food consumption or growth is shown by the results of feeding the basal diets of corn and rye both ad libitum and at the same level of food intake.

EXPERIMENTS WITH SWINE

The only rye diets fed to rats which gave poor growth were those containing ergot. These experiments indicated that ergot might be responsible for the unsatisfactory results when rye is fed to swine. Accordingly, two experiments were carried out to obtain light upon this phase of the question.

EFFECT OF 1 PERCENT OF ERGOT UPON GROWTH

In the first experiment the effect of ergot upon growth was investigated. Six pairs of pigs were used. One pig of each pair received a rye ration while its pair mate received the same amount of the same ration to which had been added 1 percent of ergot. The rye used contained only a trace of ergot. The pigs were fed twice daily in individual crates.

The basal ration consisted of rye 91.16 percent, liver meal 7.36 percent, mineral mixture 1.48 percent, plus 10 cc of cod-liver oil daily per pig.

The ergot was hand-picked from some elevator screenings. It appeared to be a mixture of ergot from the various grains. Before feeding it in this experiment it was fed to several rats as a part of a

rye diet and found to reduce greatly their food intake, as in the first of the rat experiments. The mineral mixture employed was that fed to the station swine herd, and consisted of limestone 50.00 percent, bone meal 27.97 percent, 20.00 percent salt, iron oxide 2.00 percent, copper sulphate 0.01 percent, and potassium iodide 0.02 percent.

The ration containing the ergot was very distasteful to the pigs for several weeks after the experiment began. One pig ate such small quantities that it barely maintained its weight for 4 weeks, and another ate so little that it just maintained its weight for 6 weeks. Most of the pigs seemed gradually to accustom themselves to the ergot, although there was considerable variation in this respect, as is evidenced by the growth and feed-consumption data in table 3.

TABLE 3.—*The effect of 1 percent of ergot in a rye ration upon the growth of pairs of swine over a period of 12 weeks*

[Weights in pounds]

Pair and ration	Initial weight	Final weight	Total gain	Total feed
Pair 1, males:				
Control	70.3	143.3	73.0	223
Ergot	68.6	132.6	64.0	223
Pair 2, males:				
Control	56.3	113.6	57.3	151.7
Ergot	53.6	89.3	35.7	151.7
Pair 3, males:				
Control	47.6	92.3	44.7	106.5
Ergot	46.0	68.0	22.0	106.5
Pair 4, females:				
Control	51.6	112.3	60.7	162.5
Ergot	51.0	93.3	42.3	162.5
Pair 5, females:				
Control	46.6	103.6	57.0	175.5
Ergot	48.3	95.0	46.7	175.5
Pair 6, females:				
Control	64.3	145.6	81.3	241.7
Ergot	66.0	124.0	58.0	241.7

A statistical study was made of the data in table 3 according to the method of student (1). The results were as follows:

Mean difference in total gain in weight between pair mates—pounds	17.55
Standard deviation	5.807
Z value	3.0
Probability	0.9994

That the mean difference of 17.55 pounds per pig between pair mates was due to the ergot in the ration of the experimental pig is shown by the statistical study. The probability of 0.9994 gives odds of over 1,000 to 1 that this was the case. While this experiment shows conclusively that ergot retards growth over and above any effect upon feed consumption, it does not, of course, tell to what extent feed consumption was retarded by this percentage of ergot. Throughout the experiment both pigs of each pair showed signs of hunger, and that they were hungry is indicated by the fact that the daily feed consumption was only 2.1 pounds per pig. Pigs weighing as much as those used in this experiment should eat at least twice that amount.

EFFECT OF ONE-HALF OF 1 PERCENT OF ERGOT UPON FEED CONSUMPTION

In order to determine whether aging causes ergot to lose its property of imparting distastefulness to a ration and also to determine the effect of a lower level of ergot upon feed consumption, another experiment was performed 1 year later in which the ergot was from the same supply that had been used in the first experiment.

Three pairs of pigs of an average weight of 50.6 pounds were selected for this experiment. Although they were paired as to sex, litter, and weight, both pigs of each pair were allowed to eat all the feed for which they cared twice each day. One pig received the basal ration consisting of rye 91.16 percent, liver meal 7.36 percent, and the mineral supplement 1.48 percent. Its pair mate received the basal ration plus 0.5 percent of ergot. Both pigs also received 10 cc of cod-liver oil daily. At the end of 3 weeks the rations were reversed for the same length of time. The rye used was entirely free of ergot.

TABLE 4.—*The effect of 0.5 percent of ergot in a rye ration upon the food consumption (pounds) per pig per week, of pairs of swine, over a period of 6 weeks*

Pair and ration	Food consumption					
	First week	Second week	Third week	Fourth week [1]	Fifth week	Sixth week
Pair 1, female:						
Basal	15.7	18.3	21.5	19.1	21.8	28.0
Ergot	7.1	14.2	15.4	8.9	15.1	16.5
Pair 2, male:						
Basal	10.0	14.3	15.7	15.9	27.1	26.7
Ergot	6.0	11.6	15.2	8.4	15.2	16.7
Pair 3, female:						
Basal	11.8	16.9	18.1	9.4	19.1	18.5
Ergot	7.3	10.0	10.8	7.6	9.7	8.0

[1] Rations were reversed at end of third week.

As shown by the feed-consumption data in table 4, the 0.5 percent of ergot had a marked effect upon the palatability of the ration. For the 6 weeks only 62.1 percent as much of the ergot ration was eaten as of the basal ration. Again, as in the first experiment a year earlier, there was a noticeable difference in the apparent distastefulness of the ergot to the various pigs. Also, as in the first experiment, there was a gradual tendency for the pigs to overcome their dislike for the ration containing the ergot. The average daily amount of the basal ration eaten for the 6-week period was 2.64 pounds. This daily feed consumption, which is rather low, is, however, somewhat higher than that eaten by a similar group of pigs when the trinity mixture was used as the protein supplement instead of liver meal and the feeds were self-fed instead of fed twice daily as was done in this experiment. Liver meal was fed with the rye in both ergot experiments in order to insure as satisfactory an intake of rye as possible and to determine whether or not a palatable protein supplement would overcome the distastefulness of the ergot. The results of feeding the liver meal in the swine experiments were in entire agreement with those obtained in the rat experiments.

SUMMARY AND CONCLUSIONS

The effects of ergot upon the growth and food consumption of rats and swine were investigated. To determine the effect of ergot upon the growth of rats it was fed in two paired-feeding experiments as 1.0 percent of a purified diet and at the same percentage of a diet containing 91.74 percent of rye. In the swine experiment one paired-feeding test was carried out in which the controls received a rye basal diet and the experimental pigs received the same diet in which 1 percent of the rye had been replaced by ergot. For the rats the rye diet

containing the ergot was so unpalatable that some of the rats in these paired-feeding tests, controls as well as experimentals, had died of starvation by the end of 6 weeks. Although the purified diet containing the ergot was less unpalatable than the ergotized rye diet the food intake in both experiments was at such a low level that there was little difference in growth between the rats of a pair. In the swine experiment the ergot retarded growth to a statistically significant degree.

The effect of ergot upon the food consumption of rats was studied by feeding it as 1 percent of a diet containing rye and as 1, 0.5, and 0.1 percent of diets containing rye and liver meal. Ergot from another supply was fed as 1 percent and 0.5 percent of a diet containing rye and of a diet containing corn as the cereal. One year after the first swine experiment ergot from the same supply was fed to swine as 0.5 percent of a rye ration. In these experiments with rats the first ergot that was fed retarded food consumption at all three levels, the effect diminishing as the level of ergot was decreased. The liver meal which greatly increased the consumption of the rye basal diet did not overcome the apparent distastefulness of the diets containing the ergot. In the second ergot experiment with rats the growth and food consumption, although somewhat subnormal, were surprisingly high. Ergot appears to vary considerably in the content of the principles which cause its distastefulness. There was but little difference in the food consumption and growth of the rats receiving the rye and ergot diets as compared with those receiving the corn and ergot diets. In the swine experiment the ergot had not lost its distastefulness after 1 year in storage, for at a 0.5-percent level, feed consumption was retarded.

There were no gross symptoms of ergotism in any of the experimental animals. There would seem to be little danger of this disease occurring in swine that are fed grain containing ergot because of their pronounced distaste for feeds infected with this fungus.

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FIELD REACTION OF VARIETIES AND SELFED LINES OF CORN TO DIFFERENT COLLECTIONS OF USTILAGO ZEAE[1]

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INTRODUCTION

Extensive corn-breeding investigations at different places in the United States have shown that selfed lines of corn resistant to *Ustilago zeae* (Beckm.) Ung. are rather readily isolated by inbreeding and selection (*3*, *6*, *13*).[3] The importance of developing smut-resistant varieties of corn is obvious, since the smut cannot be controlled satisfactorily by seed treatment or by cultural practice.

Recent investigations have emphasized the important role that physiologic specialization plays in the development of smut-resistant varieties of cereal crops. However, the significance of physiologic specialization in breeding smut-resistant varieties of corn is not known. Christensen and Stakman (*2*) and Eddins (*3*) have demonstrated that varieties and selfed lines of corn react differently to different collections of smut when inoculated by artificial methods. Recent studies have shown that *Ustilago zeae* comprises an indefinite number of lines or biotypes, some of which frequently give rise to variants in culture (*1*, *15*). Furthermore, it has been demonstrated that *Ustilago zeae* is predominantly heterothallic, usually requiring the pairing of two unisexual lines of opposite sex for normal infection and production of chlamydospores (*1*, *14*). There is, however, only meager information on the behavior of varieties and selfed lines of corn under field conditions when inoculated with a mixture of chlamydospores of *Ustilago zeae* from different localities. A preliminary study in Minnesota by Immer and Christensen, however, indicated that selfed lines of corn did not react differently to smut collections from University Farm and those collected in different localities throughout the Mississippi Basin (*10*).

This paper reports two additional years' data on the collections of smut previously used and data on collections obtained from a wider area.

MATERIAL AND METHODS

A representative group of approximately 100 selfed lines of corn of all gradations for resistance and susceptible to smut were selected from field, sweet, and pop-corn types. Five standard varieties of corn also were included in the tests. The same selfed lines of corn

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[2] The writers are greatly indebted to M. B. Moore, assistant in plant pathology, for assistance during the absence of the senior author in 1930. They are also indebted to C. J. Anderson, R. O. Bulger, W. Butler, R. O. Bridgford, R. A. Brink, A. M. Brunson, E. H. Coles, G. C. Curran, R. S. Dunham, H. H. Flor, I. L. Forbes, M. T. Jenkins, T. A. Kiesselbach, G. C. Mayoue, L. H. Person, H. A. Rodenhiser, L. J. Stadler, A. F. Thiel, H. L. Thomas, H. H. Thornberry, J. M. Wallace, and others for their kindness in supplying collections of smut.

[3] Reference is made by number (italic) to Literature Cited, p. 56.

were used throughout the tests, thus giving opportunity for studying seasonal fluctuations in severity of infection and the degree of constancy of reaction of these lines from year to year. Thirty plants of all lines were grown in each of two replications on each of two fields designated E and X, and separated by more than a mile. On field E an artificial smut of epiphytotic was induced by using chlamydospores collected from University Farm only; and on field X, from 1928–31, inclusive, a similar epiphytotic was induced by using a mixture of smut collected from numerous localities in the northern Mississippi Valley. In 1932 the study on field X was discontinued, and similar inoculations were made on field T, but additional collections of smut were obtained from 26 localities in 12 States east of the Rocky Mountains. In the fall smut galls were gathered from infected plants in each field and used for inoculum during the following year on the same fields. The epiphytotic was induced each year by mixing a portion of the chlamydospores with fresh barnyard manure before the manure was applied in the rows when the corn plants were from 12 to 18 inches tall. At frequent intervals during the growing season chlamydospores also were sprayed or dusted on the plants. When the corn was almost mature notes were taken on the number of infected plants and on the size, location, and number of the smut galls per plant.

EXPERIMENTAL RESULTS

The behavior of selfed lines and varieties of corn to local and nonlocal collections of *Ustilago zeae* was determined by analysis of the total percentage of smut infection, location of galls, and number and size of galls per plant.

REACTIONS OF CORN TO SMUT

The reaction of 20 representatives of 95 selfed lines of corn grown for 6 years and inoculated with chlamydospores of *Ustilago zeae* of local and nonlocal origin is given in table 1. The results indicate that most of the selfed lines used in this study were relatively homozygous for smut reaction. In general, the lines which were resistant 1 year tended to be resistant the following year, while lines susceptible 1 year proved to be susceptible the following year. The interannual correlation coefficients on the reaction of all the lines of corn grown in field E between 1930 and 1931 was 0.683; between 1931 and 1932, 0.684; and between 1932 and 1933, 0.700. These coefficients all exceed the 1-percent point (odds greater than 99:1) in significance and add further evidence of the relative homozygosity of the lines of corn to specific smut reaction.

The data presented in table 1 indicate also that selfed lines in a given year had a tendency to react alike on fields E and X or T. In all years except 1931 the average smut percentage was slightly higher on field E than on the other two fields. The differences, however, are not statistically significant. The reaction of all lines to the two collections of smut was determined by comparing the correlation coefficient between two replicates on the same field with the correlation coefficient between single replicates of the two fields. These results are summarized in table 2. Table 2 also includes the 2-year summary of the study made in 1928 and 1929, which was previously reported by Immer and Christensen (*10*).

TABLE 1.—*Smut reaction of 20 selfed lines of corn inoculated with chlamydo-spores of local and nonlocal origin, 1928–33, grown on three different fields*

Culture no.	Years selfed	Percentage of smut															
		1928		1929		1930		1931		1932		1933		Average			
		Field E [1]	Field X	Field E	Field X	Field E	Field X	Field E	Field X	Field E	Field T	Field E	Field T	Field E	Fields X and T		
	Number	Percent	Percent	Percent	Percent	Percent	Percent	Percent	Percent	Percent	Percent	Percent	Percent	Percent	Percent		
S–12	8	4	0	7	0	11	0	19	20	17	5	25	17	14	7		
S–16	9	23	5	56	22	56	39	32	33	67	33	70	57	51	32		
S–17	9	35	19	2	4	15	4	8	15	10	0	.....	.....	14	8		
S–24	6	2	0	5	2	9	0	14	11	15	11	21	14	11	6		
S–28	9	15	14	38	42	51	41	35	23	63	44	40	41	40	34		
S–32	11	23	0	21	2	5	9	44	24	13	18	.....	.....	21	11		
S–41	9	.....	.....	30	47	38	31	38	40	21	12	53	7	36	27		
S–42	8	1	0	4	0	3	0	4	2	4	3	5	0	4	1		
S–43	8	10	8	20	6	25	18	24	48	55	46	42	43	29	28		
S–54	7	.....	.....	2	2	0	0	2	2	0	8	7	4	2	3		
S–58	8	.....	.....	79	84	81	38	74	90	81	86	76	84	78	76		
S–73	7	3	7	4	0	2	4	2	2	0	3	2	3	2	3		
S–75	7	10	0	18	2	4	4	13	20	6	8	13	17	11	9		
S–76	7	13	6	15	2	10	16	15	9	19	29	.....	.....	14	12		
S–78	8	12	0	18	0	8	3	31	13	7	14	12	38	15	11		
S–80	7	9	6	23	14	48	38	37	42	39	64	35	50	32	36		
S–85	7	.....	.....	99	77	50	86	20	8	17	21	11	5	39	39		
S–88	8	0	0	47	32	31	22	92	63	70	52	44	48	47	36		
S–93	8	9	0	17	8	19	5	39	55	30	4	.....	.....	23	14		
S–100	.....	22	7	24	21	2	0	17	32	25	33	31	39	20	22		
Average	.....	12	4	26	18	23	18	28	28	28	25	30	29	25	21		

[1] Field E was inoculated with chlamydospores of *Ust. zeae* collected at University Farm, Minn.; field X was inoculated with chlamydospores obtained from the Mississippi Valley region. In 1932 field X was discontinued and similar inoculations were made on field T, but additional chlamydospore collections were obtained from wider sources.

TABLE 2.—*Correlation coefficients between percentage of smut infection of selfed lines of corn and varieties grown in two replicates in fields E and X or T, 1928–33*

Nature of correlation	Correlation coefficients					
	1928	1929	1930	1931	1932	1933
First and second replicate of field E	0.599±0.023	0.648±0.023	0.776±0.027	0.715±0.039	0.763±0.028	0.726±.033
First and second replicate of field X	.595±.023	.655±.023	.810±.023	.664±.041	.694±.036	.838±.021
Average	.597±.023	.651±.022	.794±.027	.690±.039	.733±.033	.788±.027
First replicate of field E with first replicate of field X	.350±.031	.623±.024	.679±.042	.720±.036	.673±.039	.785±.027
Second replicate of field E with second replicate of field X	.388±.030	.676±.024	.779±.027	.607±.046	.624±.042	.781±.027
Average	.374±.031	.653±.023	.709±.034	.668±.039	.652±.040	.785±.027
Average infection field E with average infection field X or T	.473±.028	.748±.018	.759±.029	.813±.037	.850±.020	.854±.019
N	358	281	100	84	92	93

An analysis of the data in table 2 clearly shows that, with the exception of the 1928 season, the correlations between two replicates within a field are essentially the same as the correlations of single replicates between the fields E and X. The average coefficients given

in table 2 and other tables were obtained by the method given by Fisher (*4, p. 164*). The average correlation coefficients between two replicates within a field were not significantly different from the average correlation coefficient of single replicates between the fields from 1929 to 1931, inclusive. These results indicate that, on an average, the lines used reacted similarly to the local and nonlocal smut collections. In 1928 there was a lower correlation between individual series of two fields as compared with the correlation between series of the same field. Immer and Christensen (*10*), however, report that the seed planted in 1928 on field X germinated 2 weeks later than that on field E, because of unfavorable conditions on the former field, and suggested this as the possible cause of differential response. The present data tend to support this conclusion.

TABLE 3.—*Comparison of 5- or 6-year average smut infection on 20 resistant lines of corn inoculated with local and nonlocal smut collections and grown on three different fields*

Culture no.	Years selfed	Years averaged	Percentage of infection		Increase(+) or decrease (−) in percentage of infection in fields X and T over that in field E
			Field E	Fields X and T	
	Number	*Number*	*Percent*	*Percent*	*Percent*
8-73	7	6	2.1	3.2	+1.1
8-54	7	5	2.8	2.6	−0.2
8-42	8	6	3.4	1.1	−2.3
8-9	8	5	3.5	4.5	+1.0
8-31	10	5	3.6	1.0	−2.6
8-15	8	6	4.9	5.8	−0.9
8-77	7	6	5.1	6.8	+1.7
8-56	7	5	7.2	9.9	+2.7
8-35	9	5	8.2	8.2	0.0
8-75	7	6	8.8	12.5	+3.7
8-85	7	6	9.1	10.9	+1.8
8-34	6	6	10.9	6.1	−4.8
8-84	7	6	11.2	11.3	+0.1
8-64	8	5	11.6	7.7	−3.9
8-72	7	5	13.1	10.6	−2.5
8-98	8	6	13.5	13.2	−0.3
8-78	8	6	14.4	11.4	−3.0
8-90	7	6	17.9	14.7	−3.2
8-26	10	5	18.3	22.7	+4.4
8-82	8	6	19.5	8.5	−11.0

Since the conclusions drawn from a study of the correlation coefficients in table 2 are based on the average reaction of a large number of lines rather than on individual lines, a further study of the data was made to determine whether there were significant differences between the reaction of individual resistant lines grown on the two fields on which local and nonlocal smut had been applied. The data of these comparisons for the 5- to 6-year period are summarized in table 3. In general, the selfed lines of corn resistant to local collections of smut on field E were equally resistant in fields X or T inoculated with a mixture of smut from many different localities. None of the differences in smut reaction between the lines listed in table 3 are statistically significant. In order to make more direct comparisons with the two different collections, resistant lines were analyzed on the basis of the periods in which the lines on field X and T were inoculated. During the 4-year period 1928 to 1931, inclusive, 26 lines, with an average

smut reaction from 1 to 10 percent, grown on field E were compared with the same lines grown on field X. None of these lines of corn had a significant higher average smut reaction when grown on field X than when grown on field E. During this period the smut reaction of another group of 26 lines, with average smut infection from 10 to 20 percent on field E, were similarly compared with their smut reaction on field X. Likewise, none of these lines reacted significantly differently on field X.

In a similar manner the smut reaction of 16 selfed lines of corn that averaged less than 10 percent of smut when grown on field E were compared with their reactions on field T in 1932 and 1933. None of these lines developed a significantly higher percentage of smut when inoculated with smut of nonlocal origin than when inoculated with the smut from University Farm collections. The maximum deviation in smut infection for a particular line occurred in 1933, when line S–78 developed 26.6 percent more smut on field T than on field E. However, the difference for the 2-year average was only 17 percent, which was 1.5 times the probable error. For the 5-year period (1929–33) the average infection on line S–78 was 3 percent more on the field E inoculated with local smut than on fields X and T inoculated with nonlocal smut.

The 2-year average smut infection during the same period of another group of 20 lines, in which the infection ranged between 10 and 20 percent on field E, was compared with their reaction on field T. There were no statistically significant differences in infection between the same lines on the two separate fields. However, in a few cases there were considerable differences in percentages of smut infection in the same line grown on fields E and T. For example, during 1932 and 1933, line S–79 developed on an average 25.5 ± 13.5 percent more smut in field T than in field X. A comparison of the specific location of the smut galls on this line in each field showed that in 1932 the increase in infection on field T was due to a greater number of infections on the suckers, and in 1933 the increase was due to a greater number of ear infections on field T. In general these results agree with those obtained previously by Immer and Christensen (10).

The effect of variation in environmental conditions on the prevalence of corn smut has been recognized (*9*). Recently, Walter[4] has accounted for fluctuation in susceptibility within a line or variety on the basis of the interaction of the growth phase of the host and environment.

The data on 61 selfed lines of corn grown on field E, X, or T from 1929 to 1933, inclusive, were used to determine the extent of variability in average smut infection that was dependent on seasonal fluctuation or on differences in reaction between fields. In 1929 and 1930 there was significantly less smut on the selfed lines grown on field X than on field E (table 4). For these 2 years there was, respectively, 11.2 ± 2.39 and 7.9 ± 2.23 percent less smut on field X than on field E, inoculated only with the smut from University Farm. In the next 3 years (1931–33) there were no significant differences in percentages of smut infection in the fields inoculated with local and nonlocal smut collections. There were also significant interannual differences in percentages of infection between years on field X but not on field E or

[4] WALTER, J. M. SOME FACTORS INFLUENCING THE DEVELOPMENT OF CORN SMUT, USTILAGO ZEAE (BECKM.) UNG. 87 pp., illus. (Unpublished doctor's thesis, Univ. Minn.)

T. For instance, the difference between the average infection on field X for years 1930 and 1931 was 8.4 ± 2.14, but for field E it was only 3.1 ± 2.48. It is, however, worthy of note that the relative susceptibility among selfed lines of corn was not materially altered, despite differences in severity of infection between fields (tables 1 and 3). Nevertheless, the results indicate that for the 6-year period (1928–33), growth and environmental factors were more instrumental in influencing the reaction of a line of corn to smut than were the different collections of smut employed. Therefore, to eliminate error due to growth and environmental conditions, comparative pathogenicity tests should be made under as nearly identical conditions as possible and over a period of years.

TABLE 4.—*Average percentage of smut infection on 61 selfed lines of corn grown on field E, X, or T, 1929–33*

Year	Total smut percentage on fields indicated		Increase (+) or decrease (−) in percentage of infection in field E over that in fields X or T	Year	Total smut percentage on fields indicated		Increase (+) or decrease (−) in percentage of infection in field E over that in fields X or T
	Field E	Field X or T			Field E	Field X or T	
	Percent	*Percent*	*Percent*		*Percent*	*Percent*	*Percent*
1929	25.9±1.88	14.7±1.48	11.2±2.39	1932	29.6±1.74	28.5±1.57	1.1±2.34
1930	25.0±1.75	17.1±1.39	7.9±2.23	1933	30.2±1.72	30.1±2.00	.1±2.64
1931	28.1±1.76	25.5±1.63	2.6±2.40				

SPECIFIC LOCATION OF SMUT GALLS

Several investigators (*5*, *6*, *7*, *9*, *10*) have found that some selfed lines of corn have a tendency to become infected on definite parts of the plant, i. e., tassel, neck (peduncle), or ear. The reason for such specificity in location of smut galls is not definitely known, although it has been suggested that physiologic forms may be partly responsible. Immer (*8*) concluded that the position of the gall most likely was associated with certain morphological characters. During 1932 and 1933 observations were made on the relative frequency of galls on ears, suckers,[5] and shoots (potential ear buds) on 84 lines of corn grown on fields E and T. These locations were selected in preference to others because of the relative frequency of gall occurrence on them. The behavior of 16 selfed lines (representative of 84 lines) inoculated with chlamydospores of local and nonlocal origin is given in table 5. The results indicate clearly that the 16 lines of corn in the given years reacted similarly with respect to specificity of position of galls on plants in the two fields. There was a greater difference between percentage of infection in specific locations in the same fields in different years than between different fields in the same year. (See cultures S–3 and S–7 in table 5.)

Statistical analysis of the data in table 6 shows that the correlation coefficients of infection of 84 lines at specific locations between 1932 and 1933 in the same fields (E and T) were no greater than between different fields. These results indicate that the different lines of corn had a tendency to become infected at the same location, whether inoculated with local or nonlocal collections of chlamydospores. The

[5] Sucker smut-galls located on any part of a stalk arising from the basal nodes.

results further indicate that the tendency for specificity of location of galls is characteristic of the lines of corn concerned.

NUMBER AND SIZE OF GALLS PER PLANT

In a similar manner, a study was made of the number and size of galls per plant on 84 selfed lines of corn grown on fields E and T. The data of this study are presented in tables 7 and 8. The correlation coefficients obtained for number of galls per infected plant on the same fields for different years were approximately of the same magnitude as those for the two fields in the same year. Apparently the different smut collections did not influence the number of smut galls that developed per infected plant.

TABLE 5.—*Comparison of the percentages of smut on 16 selfed lines of corn with infection at specific locations when grown on fields E [1] and T in 1932 and 1933*

Culture No.	Years selfed	Percentage of smut on—											
		Ear				Sucker				Shoot			
		1932		1933		1932		1933		1932		1933	
		Field E	Field T	Field E	Field T	Field E	Field T	Field E	Field T	Field E	Field T	Field E	Field T
	Number	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
8–3	9	7	5	50	42	0	2	0	2	20	30	23	37
8–7	8	0	2	28	17	3	4	8	7	16	7	14	5
8–10	9	0	0	0	0	5	0	0	0	0	0	6	16
8–14	16	0	0	13	15	32	17	22	8	5	22	19	4
8–17	9	0	0	2	0	0	0	2	2	0	0	0	0
8–25	6	10	10	12	21	37	17	14	23	22	24	20	17
8–30	11	6	2	20	35	72	36	26	45	6	2	5	30
8–38	8	11	9	34	57	0	0	0	4	19	26	43	57
8–58	8	27	29	52	64	54	39	24	48	35	25	19	24
8–66	8	2	0	2	0	25	27	2	15	16	13	4	4
8–74	8	0	0	0	0	0	0	0	0	22	16	2	11
8–88	8	2	2	0	3	2	9	2	8	66	24	40	28
8–89	8	0	0	2	0	6	10	14	8	9	13	2	3
8–92	8	0	0	0	0	33	30	11	3	4	0	0	0
8–97	7	0	0	2	0	5	4	0	5	25	11	25	10
8–100	8	0	0	19	8	4	0	0	2	15	25	23	29

[1] Field E was inoculated with chlamydospores of *Ustilago zeae* collected at University Farm, Minn., while field T was inoculated with chlamydospores obtained from many different localities east of the Rocky Mountains.

TABLE 6.—*Annual and interannual correlations of the location of smut galls on 84 selfed lines of corn grown on fields E [1] and T in 1932 and 1933*

Nature of correlation	Correlation coefficients at specific location of infection indicated		
	Shoot	Sucker	Ear
Field E, 1932 and 1933	0.741±0.033	0.645±0.043	0.596±0.047
Field T, 1932 and 1933	.564± .050	.677± .041	.800± .027
Average	.662± .041	.661± .041	.713± .036
Fields E and T, 1932	.670± .041	.769± .030	.760± .031
Fields E and T, 1933	.698± .038	.731± .034	.799± .027
Average	.684± .039	.751± .032	.781± .029

[1] See footnote 1, table 5.

TABLE 7.—*Average number of smut galls per infected plant and average size of galls on selfed lines of corn grown on fields E [1] and T in 1932 and 1933*

Culture No.	Years selfed	Galls per infected plant [2]				Average size [2] of galls [3]			
		Field E		Field T		Field E		Field T	
		1932	1933	1932	1933	1932	1933	1932	1933
	Number	*Number*	*Number*	*Number*	*Number*				
S-2	----	1.00	1.00	1.00	1.00	1.60	1.00	1.50	1.25
S-7	7	1.21	1.59	1.95	1.26	1.41	1.98	1.41	1.77
S-10	8	1.00	1.19	1.00	1.05	1.50	1.36	1.00	1.35
S-13	8	1.15	1.25	1.00	1.27	1.80	1.48	1.44	1.45
S-14	16	1.00	1.50	1.06	1.20	1.58	1.92	1.72	1.92
S-16	8	1.14	1.54	1.23	1.63	1.83	1.98	1.76	2.03
S-18	8	1.18	1.75	1.14	1.60	1.92	1.79	1.75	1.25
S-24	6	1.00	1.11	1.00	1.14	1.42	1.10	1.50	1.38
S-25	9	1.38	1.61	1.53	1.78	1.84	1.58	1.67	1.45
S-27	8	1.00	1.60	1.00	1.44	1.50	1.59	1.50	1.17
S-28	8	1.63	1.41	1.13	1.33	1.69	1.38	1.59	1.36
S-30	11	1.33	1.18	1.16	2.39	1.98	2.08	1.65	1.68
S-34	9	1.24	1.60	1.23	1.80	1.67	1.92	1.63	1.41
S-62	8	1.11	1.20	1.14	1.11	1.39	1.39	1.52	1.10
S-67	8	1.17	1.59	1.18	1.79	1.67	1.39	1.50	1.34
S-80	7	1.28	1.57	1.09	1.29	1.65	1.27	1.64	1.22
S-85	7	1.00	1.14	1.09	1.00	1.44	1.25	1.17	1.00
S-98	8	1.20	1.17	1.00	1.00	1.17	1.43	1.80	1.20

[1] See footnote 1, table 5.
[2] Based on average of 2 replicates.
[3] Smut galls are divided into 3 classes: 1=galls less than 2 inches in any diameter; 2=galls between 2 and 4 inches in diameter; 3=galls 4 inches or more in diameter.

TABLE 8.—*Annual and interannual correlation on the number of smut galls per infected plant and average size of galls on 84 selfed lines of corn grown on fields E [1] and T in 1932 and 1933*

Nature of correlation [2]	Galls per plant	Average size [3] of galls
Field E, 1932 and 1933	0.431±0.0599	0.208±0.0704
Field T, 1932 and 1933	.401± .0618	.002± .0736
Average	.417± .0608	.104± .0728
Fields E and T, 1932	.461± .0580	.117± .0726
Fields E and T, 1933	.573± .0494	.597± .0474
Average	.519± .0538	.277± .0679

[1] See footnote 1 table 5.
[2] Based on 2 replicates in each field.
[3] Gall size: 1=galls less than 2 inches in any diameter; 2=galls between 2 or 4 inches in any diameter; 3=galls 4 inches or more in any diameter.

It is evident from tables 7 and 8 that the size of galls per infected plant is of little value in making comparative studies of susceptibility between lines of corn, and consequently also between collections of smut. There was a fairly high correlation between percentage of smut infection and number of galls per infected plant, but there was no correlation between percentage of smut infection and size of gall. Thus a correlation coefficient between the average 2-year percentage of smut infection and number of galls per plant for field E was 0.672 ± 0.060 and for field T, 0.750 ± 0.042; which indicates that susceptible lines tend to have more galls per plant than resistant lines. The correlation coefficients between average size of smut galls and percentage of smut infection obtained for field E was 0.044 and for field T, 0.013.

DISCUSSION

The results of these comparative studies on the response of selfed lines of corn to mass inoculation with chlamydospores of *Ustilago zeae* from different localities in the United States under natural field conditions are of particular value in the application to breeding for smut resistance. It was clearly shown that lines of corn resistant to local collections of smut were resistant to smut collections from widely different sources. The fact that there is close agreement in smut reaction of lines of corn to smut collections from many different localities should greatly facilitate the breeding of corn for smut resistance. In fact, some progress has been made. A number of promising crosses produced from resistant or semiresistant lines of corn have proven to be more resistant than the original varieties when subjected to a similar epiphytotic of *U. zeae* obtained from widely different sources.

Christensen and Stakman (*2*), Immer (*8*), Immer and Christensen (*9*), Hoover (*7*), Walter,[6] Kyle (*11*), and others (*3, 10, 12*) have demonstrated that there are physiological, morphological, and perhaps also functional types of resistance in corn to *Ustilago zeae*. The failure of selfed lines of corn to respond in a differential manner to different collections of smut indicates that the resistant lines used in these tests possess some type of resistance to the pathogen other than the physiologic. Obviously the lines of corn tested have been exposed to infection by numerous biotypes of *Ustilago zeae*. This has been proven by numerous isolation tests. Since field E and neighboring fields have been planted to corn for many years, it is highly probable that new biotypes of smut arising from hybridization, mutation, or introduction, have had ample opportunity to persist. It seems likely, therefore, that lines of corn physiologically susceptible did not escape infection. This evidently accounts for the similarity in behavior of the selfed lines in both fields.

It has been shown by the writers and others that environmental factors influence the prevalence of smut on varieties and selfed lines of corn. It is, however, highly significant that the relative susceptibility of the particular lines tested remained constant from year to year, although the percentages of infection differed considerably. Therefore, selfed lines or crosses which are resistant at this station could probably be utilized over a fairly wide area with some assurance that they would be resistant in regions with similar climatic conditions.

To insure selection of smut-resistant lines of corn it would seem highly desirable to subject them for a period of years to as severe epidemics of smut as possible. Furthermore, in order to eliminate as far as possible physiologically susceptible lines the inoculum should be obtained from the general region in which the corn is to be grown.

SUMMARY

A study was made of the behavior of 5 varieties and 95 selfed lines of corn under natural field conditions when inoculated with smut collections of chlamydospores from University Farm, St. Paul, Minn., and collections obtained from numerous localities in 12 different States east of the Rocky Mountains.

The annual correlation coefficients between percentages of smut infection in replicates of each field with local and nonlocal smut,

[6] Walter, J. M. See footnote 4.

respectively, were essentially of the same magnitude as those between single replicates of the different fields inoculated. In general, these results indicate that the relative reactions of all the lines of corn were similar on the two fields.

Comparison of the average smut reactions of individual resistant lines grown on two fields failed to show a single instance of statistically significant difference. Lines of corn resistant to local smut collections were equally resistant to smut obtained from widely different sources east of the Rocky Mountains.

Growth and environmental factors caused greater fluctuation in prevalence of smut than did the different collections of smut, but the relative susceptibility of selfed lines of corn was not altered.

A study was made in 1932 and 1933 of smut infections at particular locations on the plant (ear, sucker, and shoot). No significant differences were found between smut reactions in different years in the same field and reactions in the same year in different fields. This indicates that lines of corn had a tendency to become infected at definite locations irrespective of collections of smut used.

In a similar manner it was shown that the different smut collections did not influence the number of smut galls that developed per infected plant. There was a high correlation between number of galls per infected plant and percentage of smut infection.

The size of smut galls cannot be used as the sole measure of resistance or susceptibility.

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PROCEDURE FOR RAPID CALCULATION OF MULTIPLE CORRELATION COEFFICIENTS [1]

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INTRODUCTION

The use of calculating machines has enabled investigators to enlarge and expand their work far beyond what would otherwise have been practicable. Those who use correlation coefficients have undoubtedly benefited greatly by the modern calculators. However, even the machine calculation of multiple correlation is a long, laborious procedure.

In using Wallace and Snedecor's machine methods [2] the writer has devised some short cuts which not only save a great deal of time and labor but give to the calculations a greater degree of accuracy.

The present paper reports these simplified methods of obtaining multiple correlations by the modern electric calculator.

CALCULATING THE MULTIPLE CORRELATION COEFFICIENT

Wallace and Snedecor [3] have given in detail the method for solving the normal equations necessary for calculating the multiple correlation coefficient. Ezekiel [4] also has given such a method. It is in these methods of solving the normal equations that the writer's short cuts are found.

In each of tables 1 to 9 progressively are shown the necessary figures or numbers from which to calculate the multiple correlation coefficient. The nine tables give a full set of numbers for the calculation of all nine multiple correlation coefficients.

[1] Received for publication Sept. 7, 1934; issued April, 1935.

[2] WALLACE, H. A., and SNEDECOR, G. W. CORRELATION AND MACHINE CALCULATION. Rev. by G. W. Snedecor. 71 pp., illus. Ames. 1931. (Iowa State Col. Off. Pub. 30, no. 4).

[3] WALLACE, H. A., and SNEDECOR, G. W. See footnote 2.

[4] EZEKIEL, M. METHODS OF CORRELATION ANALYSIS. 427 pp., illus. New York and London. 1930.

TABLE 1.—*The necessary numbers and products of the calculations to the completion of the "I" multiple correlation coefficient where nine characters are involved*

	A	B	C	D	E	F	G	H	I	S	Accuracy
A	1.0000	0.0243	0.0590	−0.0306	0.2236	0.4590	0.2941	0.1265	0.1554	2.3113	
		−.0243	−.0590	.0306	−.2236	−.4590	−.2941	−.1265	−.1554		
B		1.0000	.6264	.5754	.0528	−.0282	−.1025	.3251	−.0080	2.4653	
		.9994	.6250	.5761	.0474	−.0394	−.1096	.3220	−.0118	2.4091	
		−1.0000	−.6253	−.5765	−.0474	.0394	.1097	−.3222	.0118	−2.4106	+1
C			1.0000	.6294	.1994	−.3173	−.2848	.4446	.1399	2.4966	
			.6057	.2710	.1566	−.3197	−.2336	.2358	.1381	.8538	
			−1.0000	−.4474	−.2585	.5279	.3857	−.3893	−.2280	−1.4096	(1)
D				1.0000	−.1903	−.1735	−.1574	.1953	−.1781	1.6702	
				.5457	−.2808	.0063	.0193	−.0920	−.2283	−.0299	
				1.0000	.5147	−.0115	−.0354	.1685	.4184	.0548	+1
E					1.0000	−.0457	.0636	.3741	.6848	2.3623	
					.7627	−.0606	.0734	.2222	.4974	1.4952	
					1.0000	.0794	−.0962	−.2914	−.6522	−1.9604	(1)
F						1.0000	.7324	−.1767	−.0606	1.3594	
						.6141	.4754	−.0789	−.0474	.9632	
						1.0000	−.7741	.1285	.0771	−1.5685	(1)
G							1.0000	−.1475	.1456	1.5435	
							.4356	−.0155	.1488	.5689	
							1.0000	.0355	−.3416	−1.3061	(1)
H								1.0000	.4270	2.5684	
								.6975	.1732	.8709	
								1.0000	−.2483	−1.2482	−1
Beta	IA +.0459	IB −.0337	IC +.0172	ID −.1172	IE +.5210	IF −.3164	IG +.3504	IH +.2483			

[1] Checked to fourth place exactly.

$$R = \sqrt{0.57317101} = 0.7571$$

TABLE 2.—*The necessary numbers and products, in addition to those found in table 1, to complete the calculation of the "H" multiple correlation coefficient where nine characters are involved*

	A	B	C	D	E	F	G	I	H	S	Accuracy
I								1.0000	0.4270	2.2760	
								.4698	.1732	.6428	
								−1.0000	−.3687	−1.3683	(−4)
Beta	HA +.0737	HB +.1497	HC +.2266	HD +.0264	HE +.0684	HF +.0249	HG −.1614	HI +.3687			

$$R = \sqrt{0.36632281} = 0.6052$$

TABLE 3.—*The necessary numbers and products, in addition to those found in tables 1 and 2, to complete the calculation of the "G" multiple correlation coefficient where nine characters are involved*

	A	B	C	D	E	F	I	H	G	S	Accuracy
I							1.0000	0.4270	0.1456		
							.5206	.1679	.1488	0.8372	
							−1.0000	−.3225	−.2858	−1.6081	−2
H								1.0000	−.1475		
								.698063	−.0155	.850456	
								.6439	−.0635	.5805	
								−1.0000	.0986	−.9015	+1
Beta	GA −.0896	GB −.0817	GC −.0590	GD +.1324	GE −.0198	GF +.7859	GI +.3176	GH −.0986			

$$R = \sqrt{0.61310627} = 0.7830$$

TABLE 4.—*The necessary numbers and products, in addition to those found in tables 1 and 2, to complete the calculation of the "F" multiple correlation coefficient where nine characters are involved*

	A	B	C	D	E	I	H	G	F	S	Accuracy
I						1.0000	0.4270	0.1456	−0.0906		
						.5243	.1740	.1121	−.0474	0.7629	
						−1.0000	−.3319	−.2138	.0904	−1.4551	−2
H							1.0000	−.1475	−.1767		
							.708201	−.076551	−.0789	.726685	
							.6505	−.1138	−.0832	.4735	
							−1.0000	.1749	.0971	−.7279	−1
G								1.0000	.7324		
								.803638	.4754	1.314556	
								.7598	.4745	1.2343	
								−1.0000	−.6245	−1.6245	(1)
Beta.	FA	FB	FC	FD	FE	FI	FH	FG			
	+.3115	+.2030	−.1960	−.1010	+.0056	−.2279	+.0121	+.6245			

[1] Checked to fourth place exactly.

$$R=\sqrt{0.69260575}=0.8322$$

TABLE 5.—*The necessary numbers and products, in addition to those found in tables 1 and 2, to complete the calculation of the "E" multiple correlation coefficient where nine characters are involved*

	A	B	C	D	I	H	G	F	E	S	Accuracy
I					1.0000	0.4270	0.1456	−0.906	0.6848		
					.8487	.3189	.1599	−.0869	.4974	1.7381	
					−1.0000	−.3758	−.1884	.1024	−.5861	−2.0480	+1
H						1.0000	−.1475	−.1767	.3741		
						.772950	−.055175	−.096540	.2222	1.162386	
						.6531	−.1153	−.0639	.0353	.5092	
						−1.0000	.1765	.0978	−.0540	−.7797	(1)
G							1.0000	.7324	.0636		
							.810699	.469550	.0734	1.458394	
							.7602	.4746	−.0141	1.2208	
							−1.0000	−.6244	.0185	−1.6059	(1)
F								1.0000	−.0457		
								.618925	−.0606	.844497	
								.3074	.0026	.3100	
								1.0000	−.0084	−1.0085	+1
Beta.	EA	EB	EC	ED	EI	EH	EG	EF			
	+.1089	+.0497	+.2226	−.2659	+.5724	+.0506	−.0237	+.0084			

[1] Checked to fourth place exactly.

$$R=\sqrt{.5309791}=0.7287$$

TABLE 6.—*The necessary numbers and products, in addition to those found in tables 1 and 2, to complete the calculation of the "D" multiple correlation coefficient where nine characters are involved*

	A	B	C	I	H	G	F	E	D	S	Accuracy
I				1.0000	0.4270	0.1456	−0.0906	0.6848	−0.1781		
				.9442	.3574	.1519	−.0895	.6149	−.2283	1.7506	
				−1.0000	−.3785	−.1609	.0948	−.6512	.2418	−1.8541	+1
H					1.0000	−.1475	−.1767	.3741	.1953		
					.788452	−.058432	−.097598	.269597	−.0920	1.167424	
					.6532	−.1159	−.0637	.0369	−.0056	.5048	
					−1.0000	.1775	.0976	−.0564	.0086	−.7728	+1
G						1.0000	.7324	.0636	−.1574		
						.811383	.469772	.063420	.0193	1.457336	
						.7664	.4729	.0290	.0550	1.2653	
						−1.0000	−.6170	.0378	−.0718	−1.6509	−1
F							1.0000	−.0457	−.1735		
							.618997	−.063822	.0063	.844153	
							.3125	.0160	−.0498	.2787	
							−1.0000	−.0511	.1594	−.8918	+1
E								1.0000	−.1903		
								.907275	−.2808	1.510595	
								.5029	−.1272	.3757	
								−1.0000	.2529	−.7471	(1)
Beta	DA	DB	DC	DI	DH	DG	DF	DE			
	+.0281	+.2700	+.5150	−.1226	+.0185	+.1526	−.1465	−.2529			

[1] Checked to fourth place exactly.

$$R=\sqrt{.55361263}=0.7441$$

TABLE 7.—*The necessary numbers and products, in addition to those found in tables 1 and 2, to complete the calculation of the "C" multiple correlation coefficient where nine characters are involved*

	A	B	I	H	G	F	E	D	C	S	Accuracy
I			1.0000	0.4270	0.1456	−0.0906	0.6848	−0.1781	0.1399		
			.9757	.4111	.0986	−.1624	.6506	−.1665	.1381	1.9453	
			−1.0000	−.4213	−.1011	.1664	−.6668	.1706	−.1415	−1.9937	(1)
H				1.0000	−.1475	−.1767	.3741	.1953	.4446		
				.880249	−.149380	−.222077	.330552	.013538	.2358	1.499809	
				.7071	−.1909	−.1537	.0565	.0837	.1776	.6803	
				−1.0000	.2700	.2173	−.0798	−.1183	−.2512	−.9620	(1)
G					1.0000	.7324	.0636	−.1574	−.2848		
					.901482	.593090	.003034	−.085216	−.2336	1.128025	
					.8400	.5680	−.0475	−.0458	−.1996	1.1150	
					−1.0000	−.6762	.0565	.0545	.2376	−1.3274	−2
F						1.0000	−.0457	−.1735	−.3173		
						.787767	−.146465	−.136740	−.3197	.393432	
						.3433	.0062	−.1153	−.1232	.1110	
						−1.0000	−.0180	.3358	.3587	−.3233	−2
E							1.0000	−.1903	.1994		
							.947756	−.210784	.1566	1.731302	
							.5066	−.1070	.0413	.4409	
							−1.0000	.2111	−.0815	−.8703	−1
D								1.0000	.6294		
								.666942	.2710	.352080	
								.5648	.2300	.7946	
								−1.0000	−.4073	−1.4098	−5
Beta	CA	CB	CI	CH	CG	CF	CE	CD			
	+.1270	+.3273	+.0142	+.1262	−.0539	−.2249	+.1675	+.4073			

[1] Checked to fourth place exactly.

$$R=\sqrt{.64707443}=0.8044$$

TABLE 8.—*The necessary numbers and products, in addition to those found in tables 1 and 2, to complete the calculation of the "B" multiple correlation coefficient where nine characters are involved*

	A	I	H	G	F	E	D	C	B	S	Accuracy
I		1.0000	0.4270	0.01456	−0.0906	0.6848	−0.1781	0.1399	−0.0080		
		.9759	.0473	.0999	−.1619	.6501	−.1733	.1307	−.0118	1.9168	
		−1.0000	−.4174	−.1024	.1659	−.6662	.1776	−.1339	.0121	−1.9641	−2
H			1.0000	−.1475	−.1767	.3741	.1953	.4446	.3251		
			.983998	−.184704	−.234763	.345815	.199171	.437136	.3220	2.276021	
			.8140	−.2264	−.1672	.0745	.2715	.3826	.3269	1.4759	
			−1.0000	.2781	.2054	−.0915	−.3335	−.4700	−.4016	−1.8132	+1
G				1.0000	.7324	.0636	−.1574	−.2848	−.1025		
				.913505	.597408	−.002161	−.148401	−.302152	−.1096	.863747	
				.8403	.5675	−.0480	−.0552	−.2091	−.0175	1.0779	
				−1.0000	−.6753	.0571	.0656	.2489	.0208	−1.2828	−1
F					1.0000	−.0457	−.1735	−.3173	−.0282		
					.789319	−.148332	−.159455	−.344381	−.0394	.298513	
					.3449	.0072	−.0952	−.1029	.0376	.1918	
					−1.0000	−.0210	.2759	.2984	−.1090	−.5560	+3
E						1.0000	−.1903	.1994	.0528		
						.950003	−.183458	.186208	.0474	1.845493	
						.5072	−.0940	.0543	.0236	.4910	
						−1.0000	.1858	−.1072	−.0465	−.9681	−3
D							1.0000	.6294	.5754		
							.999064	.631205	.5761	1.740926	
							.8304	.4948	.4786	1.8037	
							−1.0000	−.5958	−.5763	−2.1721	(1)
C								1.0000	.6264		
								.996519	.6250	2.360233	
								.4158	.1921	.6081	
								−1.0000	−.4621	−1.4626	+5
Beta	BA	BI	BH	BG	BF	BE	BD	BC			
	−.1343	−.0394	+.1175	−.1051	+.3288	+.0527	+.3010	+.4621			

[1] Checked to fourth place exactly.

$$R = \sqrt{0.50218895} = 0.7087$$

TABLE 9.—*The necessary numbers and products of the calculations to the completion of the "A" multiple correlation coefficient where nine characters are involved*

	I	H	G	F	E	D	C	B	A	S	Accuracy
I		0.4270	0.1456	−0.0906	0.6848	−0.1781	0.1399	−0.0080	0.1554	2.2760	
		−.4270	−.1456	.0906	−.6848	.1781	−.1399	.0080	−.1554		
		1.0000	−.1475	−.1767	.3741	.1953	.4446	.3251	.1265	2.5684	
H		.8177	−.2097	−.1380	.0817	.2713	.3849	.3285	.0601	1.5965	
		−1.0000	.2564	.1688	−.0999	−.3318	−.4707	−.4018	−.0736	−1.9525	−1
G			1.0000	.7324	.0636	−.1574	−.2848	−.1027	.2941	1.5435	
			.9250	.7102	−.0152	−.0619	−.2065	−.0171	.2869	1.6215	
			−1.0000	−.7678	.0164	.0668	.2232	.0185	−.3101	−1.7528	(1)
F				1.0000	−.0457	−.1735	−.3173	−.0282	.4590	1.3594	
				.4232	.0418	−.0963	−.0813	.0397	.2629	.5901	
				−1.0000	−.0988	.2276	.1921	−.0937	−.6213	−1.3944	+3
E					1.0000	−.1903	.1994	.0528	.2236	2.3623	
					.5185	−.0866	.0698	.0213	.0895	.6125	
					−1.0000	.1677	−.1346	−.0410	−.1734	−1.1813	(1)
D						1.0000	.6294	.5754	−.0306	1.6702	
						.8376	.5066	.4764	.0712	1.8913	
						−1.0000	−.6041	−.5688	−.0851	−2.2580	(1)
C							1.0000	.6264	.0590	2.4966	
							.4225	.1860	.0084	.6770	
							−1.0000	−.4403	−.1619	−1.6024	+2
B								1.000	.0243	2.4653	
								.5102	−.0922	.4178	
								−1.0000	.1808	−.8188	−4
Beta	AI	AH	AG	AF	AE	AD	AC	AC	AB		
	+.0721	+.0779	−.1552	+.6788	+.1554	+.0420	+.2415	+.2415	−.1808		

[1] Checked to fourth place exactly.

$$R = \sqrt{0.33030087} = 0.5745$$

THE CALCULATION

Following Wallace and Snedecor's method of solving normal equations, the headings of the columns, table 1, are from A to I and the sum column. To the left are the blocks from A to H, inclusive. Each block has 4 lines, except the A block, which needs only 2. Of the 4 lines, only 3 have any use in the first table and the last (table 9) in the calculation of a full set of multiple coefficients. In line 1 are entered the simple correlation coefficients which have previously been calculated. Line 2 of the first block (block A in table 1) carries the simple coefficients of that block, with the sign changed. Line 3 of the other blocks carries the sum of the columns where it occurs, and line 4 the quotients of the sums in line 3 divided by the first sum in each block. These are the only figures necessary to enter in the table, as they are the only ones used to calculate the rest of the table below, the partial regression coefficients (the betas), and the multiple correlation coefficients.

In obtaining line 3 of the second block (B of table 1) only one multiplication is to be made in each column; in the C block, two multiplications; and for each succeeding block one more multiplication. Taking block B and column B, for example, put 1.0000 on the calculator on the left column of the keyboard. Shift dials to the right and enter the 1.0000 in the lower dial. Then mark the decimal place on the lower dial with a marker, and this place will always be the same throughout all calculations in the multiple correlation tables. At the beginning of each new operation the decimal points of the keyboard and the lower dial must be alined before the number is placed on the machine. On the writer's machine, a 1929 8-bank model, the decimal is between the fourteenth and fifteenth places on the lower dial.

After the 1.0 is entered on the machine in the proper place, one of the multipliers is entered on the keyboard. In table 1, this multiplier (line 1, block A, column B) is 0.0243, and is entered on the keyboard where the decimal is one place farther to the right than the decimal on the lower dial. This arrangement allows the multiplication to go forward, and by using the first place, left to right, on the upper left dials, there will be eight places covered on the lower dial to the right of the decimal point when both multipliers have four decimal places. Of course, the decimal place in the upper dials will be to the left of the first place. However, this multiplier can be placed on the keyboard with the decimal place the same as was the coefficient, but before multiplying the carriage must be shifted to the left one place.

Now, after 0.0243 is entered on the keyboard the multiplication with −0.0243 (block A, column B, line 2) can be made by using the subtraction switch. (The −0.0243 will be one of the multipliers for all multiplications in the calculation of the B block.) In other words, the multiplying can be done by subtracting from 1.0000. The result is 0.9994, which is entered in line 3, column B, block B, and is the number to be used to divide into all other figures of line 3 of B block to get line 4. Now, clear the machine of all figures in dials and keyboard. Enter in the lower dials the BC coefficient of line 1, column C, block B, which is 0.6264. Clear the keyboard and enter on it 0.0590 line 1, column C, block A, and multiply by subtracting −0.0243, which leaves 0.6250. This is entered on the table in line 3, column C. Clear the top dial and keyboard only and immediately divide by 0.9994 and enter the quotient, 0.6253, in line 4, column C and block B. Clear the machine and enter on the lower dial the BD coefficient, 0.5754; looking just above the BD coefficient in line 1, block A, take −0.0306 and enter it on the keyboard and multiply it by −0.0243 by using the plus switch. Adding the product to 0.5754 gives 0.5761, which goes in line 3, column D, block B. This figure is on the machine ready to be divided by 0.994 when the top dials and keyboard are cleared. The latter calculation gives 0.5765 and is entered in line 4, column D, block B, with its sign changed. This process goes on, taking up each coefficient of block B and subtracting from or adding to the product of the figure in line 1 of block A, of the same column as the coefficient, with the figure in line 2 of column B of block A. The sum is taken in the same way as the coefficients. The check figure, of course, is always line 4, column S, of each block and should equal everything to its left in the same line.

In block C there will be two multiplications added to or subtracted from the coefficients of line 1. Take column C, block C, for instance. Enter 1.0000 on the machine as before; in block A, column C, there is found 0.0590 and −0.0590 to be multiplied together and subtracted at the same time from 1.0000. Then in block B, lines 3 and 4 of column C, there are 0.6250 and −0.6253, which will be handled in a similar manner after clearing the top dials and keyboard. This leaves, after these two multiplications, 0.6057, which when divided by itself gives −1.0000. This goes in line 4 with 0.6057 just above in line 3. The next procedure is to clear the machine of all numbers. Then enter 0.6294, the coefficient CD, in the proper place on the lower dial and multiply by addition −0.0306, line 1, block A, of the same

column as the coefficient CD, by −0.0590, line 2, column C, block A. Line 4 in each block just above the first column of the block being calculated is always one of the multipliers throughout that block. The other multiplier is in each block above the coefficient, but in line 3. Continuing with column D, block C, after the A block multiplication has been made and added to the 0.6294, the next multiplication will be found in the next lower block as denoted above; which is 0.5761 and −0.6253; leaving when subtraction by multiplication is done, 0.2710. Then dividing by 0.6057 without clearing the lower dial gives −0.4474 when the sign is changed.

One more illustration will probably clarify all the details of the calculation. Passing to block H, first take column H and put 1.0000 on the lower dial; then make the following multiplications, starting by subtracting each from the number on the lower dial: (0.1265×−0.1265) of block A; (0.3220×−0.3222) of block B; (0.2358×−0.3893) of block C; (−0.0920×0.1685) of block D; (0.2222×−0.2914) of block E; (−0.0789×0.1285) of block F; and (−0.0155×0.0355) of block G. The remainder is 0.6975, which is entered in line 3, column H, block H, with −1.0000 just below in line 4.

Then, taking the next coefficient in block H, which is HI or 0.4270, and entering it on the cleared machine, the following multiplications are made, off or on according to the sign: (0.1554×−0. 1265) of block A; (−0.0118×−0.3222) of block B; (0.1381×−0.3893) of block C; (−0.2283×0.1685) of block D; (0.4974×−0.2914) of block E; (−0.0474×0.1285) of block F; and (0.1488×0.0355) of block G. The remainder, 0.1732, is entered in line 3, column I, block H. While still on the machine it is divided by the first number in line 3 of block H, which is 0.6975. This leaves −0.2483 when the sign is changed.

There is one point that may confuse some operators of calculating machines. It frequently happens in subtracting and multiplying at one operation, that the product of the multiplication is larger than the number from which it is being subtracted. When the bell rings, indicating that the former is larger than the latter, proceed with the multiplication until it is completed. When it is finished the complement of the number than on the machine can easily be obtained, and by adding it to the number and clearing the machine the complement is checked. When the complement clears the machine it can be entered immediately on the machine, and the usual division can be made. Should the number that is being subtracted from be exhausted before all multiplications are completed, the other multiplications can be made before it is necessary to know the result.

THE GENERAL SET-UP

If all possible multiple correlation coefficients are desired, skeleton tables for all variables should first be made out. The skeleton tables should then be filled in with the simple correlation coefficients and the sum of each block entered in the proper place. In the calculation of the first table there are certain numbers that can be used in some of the other tables. After completing the first two blocks there is a place in one of the tables for each multiplication when it is completed. This is the reason for the second line in each block. When calculation is being performed in tables where line 2 is filled in it is only necessary to pick up the number in line 2 and complete the multipli-

cations in the table above the block in question to finish that particular column and block. To illustrate the use of line 2, take block C, column C, of table 1; enter 1.0000 in the lower dial, multiply and subtract 0.0590×-0.0590, and record the remainder (0.996519) in line 2, column C, block C, of table 8. To illustrate this further, take in table 1, column H, block G, the coefficient in line 1, -0.1475, and enter it on the lower dial. Then the remainder (-0.184704) of the first multiplication (0.1265×-0.2941) by addition (the coefficient in negative) is placed in table 8, line 2, column G, block H. Now, clear the top dials and keyboard and make the B block multiplication (0.3220×0.1097) (by subtraction). The remainder (-0.149380) (on the lower dial) is placed in table 7, column G, block H, line 2. The multiplication in block C (table 1) is now made, which remainder (-0.058432) is entered in table 6, column G, block H. The block D multiplication is then completed and the remainder (-0.055175) recorded in table 5, column G, block H. Then the remainder of block E multiplication goes in table 4, column G, block H; and the block F multiplication remainder, of course, is recorded in line 3, column H, block G, of table 1.

In any of the calculations of the first table involving column I (the primary of first table) or block I of table 2, the remainders are recorded in line 3 of the tables, blocks, and column where they belong. In the tables other than tables 1, 2, and 9, all sums of block I which are so recorded in line 3 are complete and ready for division by the division number of that block without further multiplication. A glance at the tables will make this evident. Line 3 in the I blocks and line 2 of all other blocks of the last column (not sum column) of each table, other than tables 1 and 9 and columns I and S, block I of table 2, can be obtained by taking them direct from table 1. For instance, -0.2283 in table 6, column D, block I, occurs in line 3, column I, of block D in table 1. Also, passing down column D of table 6 on line 2, read -0.0920, 0.0193, 0.0063, -0.2808. Then look in table 1, block D, and read from right to left on line 3 the same figures in their respective columns.

CALCULATION OF TABLES OTHER THAN 1 AND 9

It must be remembered that the multiple coefficient of the last table of a series of multiple coefficients (table 9 here) must be calculated as a whole and separately from the other tables. In other words, there are no calculations in the other tables that can be used in its calculation. As previously stated, certain calculations from table 1 can be used for the other tables (tables 2 to 8 herein). These remainders of multiplications subtracted from or added to a coefficient were placed in line 2 of the corresponding block and columns where they belong. The exception to this, as has been noted, occurs in all the "I" blocks, where such sums were placed in line 3 of their columns.

To illustrate the calculation of the tables other than 1 and 9, table 4 will be used. Line 3 of block I and line 2 of blocks H and G have been filled in from calculation of table 1, with the exception of column F. In column F, line 3 of block I and line 2 of blocks H and G can be written in from block F of table 1; -0.0474 column I, -0.0789 column H, and 0.4754 column G. Then, divide line 3, block I, through by 0.5243, obtaining line 4. Next, block H: Enter the sum of HH of

line 2 (0.708201) on the keyboard, align the decimal points, transfer to the lower dials, enter 0.1740 (line 3 IH) on the keyboard one place to the right of the coefficient decimal place (or shift carriage one place to the left), and multiply by −0.3319 by subtraction. The result is 0.6505, which is entered in line 3, column H, block H; enter −1.000 just beneath.

The other columns and blocks are calculated similarly. The only difference is the entering on the machine of the number in line 2 instead of the coefficient in line 1; and with the completion of the calculations of each table as outlined, the figures necessary to calculate the partial regression coefficients and the multiple coefficient are obtained.

CALCULATION OF THE PARTIAL REGRESSION COEFFICIENTS

In the calculation of the partial regression coefficients or the betas' it is seen that these betas are placed in certain columns. In table 1, I being used as the primary, the "I" betas are found. Beta IH is in column H, IG in column G, etc. In the other tables the betas appear in their corresponding columns. Table 1 may be used to illustrate the method of calculating the betas. One beta (IH) comes out at the completion of the main body of the table as the first unknown that is found. The only change is to reverse the sign. Therefore, beta IH, +0.2483, is entered in the beta line in column H. To get beta IG, enter on the calculator (with reverse sign) the number found in line 4, column I, block G; which in this case is 0.3416. Then multiply the figure 0.0355 in block G, column H, line 4, with beta IH, 0.2483, by addition or by using the plus switch. The beta IG is then read direct from the calculator. To arrive at beta IF, use line 4 in block F. Starting with column I, enter on the machine −0.0771 and multiply 0.1285×0.2483 by subtraction; then -0.7741×0.3504 by addition and read beta IF direct from the machine. Beta IF is then entered in column F. The other betas are calculated in a similar manner, using the numbers in line 4 in each block corresponding to the beta desired and entering first the number in column I (the primary column on the machine with reversed sign and multiplying by subtraction or addition the figures in each column of line 4 with the corresponding beta previously calculated.

In calculating betas in the tables other than 1 and 9, it is necessary to use certain parts of table 1. For illustration, take table 6; beta DI is calculated from the I block of table 6, but to get DC it is necessary to go to block C of table 1. Then, entering −0.4474, which appears in column D, line 4, on the calculator with the reverse sign, take the other line 4 numbers in block C and multiply them, by subtraction or addition as their sign dictates, by their corresponding betas; the number in column E by beta DE; column F, by beta DF; column G, by beta DG; column H, by beta DH; and column I by beta DI. The beta DC is then read directly from the machine. All the other partial regression coefficients can be obtained in a similar manner.

The accuracy of the calculations of each block is indicated in the last column of each table as the difference in fourth decimal place to check figure.

The other operations, such as checking the betas by means of substituting in one of the equations and the calculation of R, are the same as given in Wallace and Snedecor (2).

The time required to calculate a set of nine multiple tables like tables 1 to 9 is about 10 hours. This does not take into account certain errors that have to be corrected that may greatly delay the completion of the calculation.

SUMMARY

Certain methods of machine calculation of multiple correlation coefficients are reported.

In the multiplication by subtracting from or adding to another number and carrying the totals on the machine until time to divide, and at that time having the number in a position where it can be divided without putting it in or taking it out of the machine, makes possible the short cut in methods described here.

These short methods of calculation reduce the time necessary to obtain the multiple correlation coefficients and give a higher degree of accuracy to the work.

SEROLOGICAL STUDIES OF SWINE ERYSIPELAS WITH PARTICULAR REFERENCE TO AGGLUTINATION [1]

By H. W. SCHOENING, *chief*, and G. T. CREECH, *veterinarian, Pathological Division, Bureau of Animal Industry, United States Department of Agriculture*

INTRODUCTION

When the results of previous investigations [2] on the agglutination test for swine erysipelas were reported, information was not available as to the time of the appearance and stability of agglutinins in the blood stream of swine following infection. To obtain data on this point, a series of observations and agglutination tests on artificially infected swine was made. Information on the subject also has recently been received from the field. These data are reported in the present paper, together with other experimental data obtained from the agglutination tests of blood serum from swine and from persons who were suspected of being infected with *Erysipelothrix rhusiopathiae*. A rapid serological method of identifying cultures of *E. rhusiopathiae* is also reported.

TIME OF APPEARANCE AND STABILITY OF AGGLUTININS IN THE BLOOD OF ARTIFICIALLY INFECTED PIGS

EXPERIMENTAL PROCEDURE

Eight pigs were used in the experiment made to determine the time of appearance and stability of agglutinins in the blood stream of artificially infected swine. Two of the pigs were 3 weeks of age, and the remainder were from 3 to 4 months of age.

All the pigs were exposed to swine-erysipelas infection by injection of 20 cc of broth cultures of *Erysipelothrix rhusiopathiae*, either intravenously, subcutaneously, or intraperitoneally, or by combinations of these methods. Before exposure, samples of serum were drawn and the rapid whole-blood and serum agglutination tests were made for swine erysipelas. All these tests were negative.

Beginning about 3 days after the exposure of the pigs to infection blood was drawn and samples of serum were collected daily for several days, carbolized 0.5 percent, and stored in the ice box for use in the tube test. The rapid plate test was made with the whole blood and serum at the time of collection of the samples. Later, samples were collected at 2- or 3-day intervals, in most instances, and tests were made as stated above. Toward the end of the experiment, bleedings were made at lengthening intervals of from 1 to 3 weeks. All the serum samples collected on the various dates were then tested against the same antigen by means of the tube test, the technic of which has already been described.[3]

[1] Received for publication Aug. 22, 1934; issued April, 1934.

[2] SCHOENING, H. W., and CREECH, G. T. AN AGGLUTINATION TEST FOR SWINE ERYSIPELAS. Jour. Amer. Vet. Med. Assoc. (n. s. 35) 82: 503-509. 1933.
——CREECH, G. T., and GREY, C. G. A LABORATORY TUBE TEST and A WHOLE-BLOOD RAPID AGGLUTINATION TEST FOR THE DIAGNOSIS OF SWINE ERYSIPELAS. North Amer. Vet. 13 (12): 19-25. 1932.

[3] SCHOENING, H. W., CREECH, G. T., and GREY, C. G. See footnote 2.

RESULTS

Six of the pigs became noticeably ill following exposure, but all recovered. Two of the pigs, nos. 4259 and 4260, 3 weeks of age, failed to show any clinical evidence of infection after exposure, and tests of their blood remained consistently negative.

The results of the various tests on each of the six pigs which showed clinical evidence of infection are recorded in table 1. With the exception of the preinfection bleedings, the table records only the results of those tests made after the first indications of reactions were noted.

TABLE 1.—*Results of agglutination tests* [1] *for swine erysipelas of individual pigs inoculated by various methods with 20 cc of a bouillon culture of Erysipelothrix rhusiopathiae*

PIG 4253, INOCULATED FEB. 25, 1933, INTRAVENOUSLY, SUBCUTANEOUSLY, AND INTRAPERITONEALLY

Date of drawing blood	Results of plate test [2] of—		Results of tube test [3] of blood serum at dilutions of—						Remarks
	Whole blood	Blood serum	1:25	1:50	1:100	1:200	1:500	1:1,000	
Feb. 25	−	−	−	−	−	−	−	−	Blood drawn prior to inoculation.
Mar. 3	(?)	+	+	+	+	+	−	−	Temperature of 104.5° F., slight depression, off feed.
Mar. 6	+	+	+	+	+	+	−	−	Temperature of 104.7°, recovery of appetite, no symptoms.
Mar. 9	+	+	+	+	+	+	−	−	Temperature of 104.2°, no symptoms.
Mar. 13	+	+	+	+	+	−	−	−	Temperature of 104°, no symptoms.
Mar. 22	+	+	+	P	S	−	−	−	Temperature of 102°, serum sample decomposed.
Mar. 29	+	+	+	+	+	−	−	−	Temperature normal on this and subsequent dates.
Apr. 11	−	−	P	VS	−	−	−	−	
May 9	−	−	P	VS	−	−	−	−	
May 25	−	−	+	P	−	−	−	−	
June 11	−	−	+	P	−	−	−	−	

PIG 4254, INOCULATED FEB. 25, 1933, INTRAVENOUSLY

Feb. 25	−	−	−	−	−	−	−	−	Blood drawn prior to inoculation.
Mar. 3	+	+	+	+	+	+	−	−	Temperature of 106.5° F., marked depression, off feed.
Mar. 6	+	+	+	+	+	+	S	−	Temperature of 104.5°, recovery of appetite, evidence of arthritis in all 4 feet.
Mar. 9	+	+	+	+	+	+	−	−	Temperature of 105.4°, well-marked arthritis.
Mar. 13	+	+	+	+	+	−	−	−	Temperature of 104.6°, swelling of joints still noticeable.
Mar. 22	+	+	+	+	−	−	−	−	Temperature of 104.1°, joints appeared normal.
Mar. 29	+	+	P	S	−	−	−	−	Normal after this date, serum sample decomposed.
Apr. 11	+	+	−	S	−	−	−	−	
Apr. 25	+	+	+	+	−	−	−	−	
May 9	+	+	+	P	−	−	−	−	
May 25	+	+	P	S	−	−	−	−	
June 16	+	+	P	S	−	−	−	−	

[1] Key: + = complete agglutination; P = partial agglutination; S = slight agglutination; VS = very slight agglutination; − = no agglutination; (?) = result questionable.
[2] Plate tests were made on same date blood was drawn.
[3] All tube tests were made on June 21 with the same lot of antigen.

TABLE 1.—*Results of agglutination tests for swine erysipelas of individual pigs inoculated by various methods with 20 cc of a bouillon culture of Erysipelothrix rhusiopathiae*—Continued

PIG 4255, INOCULATED MAR. 31, 1933, INTRAVENOUSLY AND INTRAPERITONEALLY

Date of drawing blood	Results of plate test of—		Results of tube test of blood serum at dilutions of—						Remarks
	Whole blood	Blood serum	1:25	1:50	1:100	1:200	1:500	1:1,000	
Mar. 31	−	−	−	−	−	−	−	−	Blood drawn prior to inoculation.
Apr. 3	−	+	−	−	−	−	−	−	Temperature of 105.4° F., off feed, depression.
Apr. 4	−	+	+	+	+	VS	−	−	Temperature of 105.4°, depression.
Apr. 5	+	+	+	+	+	S	−	−	Temperature of 105°, appeared brighter, ate fairly well.
Apr. 6	+	+	+	+	+	+	−	−	Temperature of 105.4°, appeared brighter, eating.
Apr. 7	+	+	+	+	+	+	−	−	Temperature of 104.6°.
Apr. 10	+	+	P	+	+	+	−	−	Temperature of 103.6°.
Apr. 17	+	+	+	+	+	P	−	−	Normal after this date.
Apr. 24	+	+	+	+	+	P	−	−	
May 9	+	+	+	+	+	VS	−	−	
May 25	+	+	+	P	−	−	−	−	
June 16	?	+	+	S	−	−	−	−	

PIG 4256, INOCULATED MAR. 31, 1933, INTRAVENOUSLY AND INTRAPERITONEALLY

Mar. 31	−	−	−	−	−	−	−	−	Blood drawn prior to inoculation.
Apr. 3	−	−	+	VS	−	−	−	−	Temperature of 106.6° F., depression, off feed.
Apr. 4	−	+	+	+	−	−	−	−	Temperature of 106.2°, depression, lame right front leg.
Apr. 5	+	+	+	+	+	+	−	−	Temperature of 105.8°, ate fairly well, lame in all 4 feet.
Apr. 6	+	+	+	+	+	+	P	−	Temperature of 106°, still lame.
Apr. 7	+	+	+	+	+	+	+	S	Temperature of 105.8°, lameness not so pronounced.
Apr. 10	+	+	+	+	+	+	+	+	Temperature of 104°, only slight lameness.
Apr. 17	+	+	+	+	+	+	S	−	Normal after this date.
Apr. 24	+	+	+	+	+	P	S	−	
May 9	+	+	+	+	+	S	−	−	
May 25	+	+	+	+	+	S	−	−	
June 16	?	+	+	+	P	S	−	−	

PIG 4257, INOCULATED APR. 27, 1933, INTRAVENOUSLY

Apr. 27	−	−	−	−	−	−	−	−	Blood drawn prior to inoculation.
May 1	−	+	+	P	−	−	−	−	Temperature of 106.2° F., depression.
May 3	+	+	+	+	?	−	−	−	Temperature of 105°, off feed.
May 5	+	+	+	+	+	+	P	−	Temperature of 104.8°, lame in all 4 feet, joints hot, swollen, and painful.
May 6	+	+	+	+	+	+	S	−	Temperature of 105°, lame in all 4 feet, joints hot, swollen, and painful.
May 15	+	+	+	+	+	S	−	−	Temperature normal, little evidence of lameness.
May 25	+	+	+	+	+	S	−	−	Knee and hock joints slightly enlarged.
June 16	+	+	+	+	+	S	−	−	Do.

PIG 4258, INOCULATED APR. 27, 1933, INTRAVENOUSLY

Apr. 27	−	−	−	−	−	−	−	−	Blood drawn prior to inoculation.
May 1	−	−	+	S	−	−	−	−	Temperature of 106° F., no visible symptoms.
May 3	+	+	+	+	+	+	−	−	Temperature of 106.5°, depression, off feed.
May 5	+	+	+	+	+	+	−	−	Temperature of 103.5°, ate fairly well, slightly lame.
May 6	+	+	+	+	+	P	−	−	Temperature of 103.5°, lame, swelling of knee and hock joints.
May 15	+	+	+	+	P	−	−	−	Normal after this date.
May 25	+	+	+	+	P	−	−	−	
June 16	(?)	+	+	+	P	−	−	−	

DISCUSSION

The first symptoms of the disease in the pigs were observed from the second to the third day after inoculation and were usually well marked on the fourth day. Agglutinins were first detected in the blood of the infected pigs from the third to the sixth day after inoculation.

Pigs 4255 and 4256 gave positive reactions to both the whole-blood plate test and the tube test 2 days after the first symptoms were observed, whereas the other 4 infected pigs did not give definite positive reactions to both tests until the third day after the first evidence of the disease.

These observations indicate that agglutinins make their appearance early in the disease, within a few days after the animal shows some clinical evidence of infection. Field observations by C. F. Harrington,[4] a veterinary inspector of the Bureau of Animal Industry, in outbreaks of swine erysipelas in South Dakota, confirm these findings. In a number of cases he applied the whole-blood plate test to animals sick but a short time and obtained positive results in some animals that were sick for only 2 or 3 days.

It is of interest to note the rapidity with which the agglutinins are formed in the blood after a certain stage of the disease. For example, pigs 4253 and 4254 gave entirely negative results to the plate and tube tests for swine erysipelas on the fifth day after inoculation, but 24 hours later they gave strong reactions to the plate test and definite positive reactions to the tube test also.

Table 1 shows the high points of the reactions to the tube tests in the different experimental pigs, the highest reactions usually occurring about the second week after infection had taken place. There was a gradual loss of reactions after the second week.

The results seem to indicate that pigs suffering only slight attacks of the disease, or those which make a prompt recovery, probably lose their reactions more or less after a comparatively short time. This finding is in keeping with field observations in which animals that had recovered from the disease without showing any marks of it gave negative reactions to the test, whereas animals in the same herd with arthritis gave positive reactions. Available data on serological tests of field cases show that pigs which have developed chronic lesions, particularly cases of chronic arthritis, will continue to give well-marked reactions for an indefinite period.

AGGLUTINATION TESTS OF SWINE WITH THE URTICARIAL FORM OF SWINE ERYSIPELAS (DIAMOND SKIN DISEASE)

In 1933 a sample of blood serum and a specimen of skin, from the same pig, showing typical diamond skin lesions were received from G. W. Stiles, Jr., in charge of the branch Pathological Laboratory of the United States Department of Agriculture at Denver, Colo. *Erysipelothrix rhusiopathiae* was recovered from the specimen of skin, but the serum sample gave negative results to both the plate and tube agglutination tests for swine erysipelas.

Stiles [5] has reported five cases of typical diamond skin disease in swine, detected on post-mortem examination at federally inspected

[4] Personal communication.

[5] STILES, G. W., and DAVIS, C. L. SWINE ERYSIPELAS AND ITS ECONOMIC IMPORTANCE. Jour. Amer. Vet. Med. Assoc. (n. s. 37) 84: 895-906, illus. 1934.

establishments, in which negative reactions to the plate agglutination test were obtained. In these cases, however, bacteriological examination of the affected skin or other tissues or both failed to disclose *Erysipelothrix rhusiopathiae*. The reason for the negative serological results in these cases of skin lesions has not been determined.

PASSIVE TRANSFER OF AGGLUTININS TO SWINE INJECTED WITH ANTI-SWINE-ERYSIPELAS SERUM

In an investigation of an outbreak of disease in a swine herd in South Dakota, C. H. Hays, inspector in charge of the Federal field station at Pierre, S. Dak., obtained a strong positive reaction to the whole-blood plate agglutination test for swine erysipelas in the case of a sick hog which died the following day. The clinical observations, post-mortem examination, and laboratory studies revealed the disease in this herd to be a suipestifer infection with no evidence of erysipelas infection whatsoever. However, this hog was in a herd which was used for studying the efficacy of anti-swine-erysipelas serum in the field, and the animal, when taken sick, had been given two injections of 25 cc each of the serum about a week previous to the test. Since it had been determined that there was no erysipelas infection in this hog, Hays raised the question whether the positive reaction in the animal was due to the injection of the anti-swine-erysipelas serum. To obtain information on this point, pig 1, weighing 20 pounds, was injected subcutaneously with 30 cc of anti-swine-erysipelas serum of equine origin. Pig 2, about the same age, size, and weight as pig 1, was given an injection of 30 cc of normal horse serum subcutaneously. Table 2 gives the results of the plate and tube agglutination tests on samples of serum drawn on different days from these animals.

TABLE 2.—*Results of agglutination tests* [1] *for swine erysipelas of pigs injected subcutaneously with anti-swine-erysipelas serum (horse) or normal horse serum*

Date of drawing blood	Results of plate test [2] of—		Results of tube test [3] of blood serum at dilutions of—						
	Whole blood	Blood serum	1:25	1:50	1:100	1:200	1:500	1:1,000	1:2,000
PIG 1, INJECTED OCT. 5, 1933, WITH 30 CC ANTI-SWINE-ERYSIPELAS SERUM (HORSE)									
Oct. 5 [4]	−	−	−	−	−	−	−	−	−
Oct. 6	+	+	+	+	+	+	+	+	−
Oct. 7	+	+	+	+	+	+	+	+	−
Oct. 9	+	+	+	+	+	+	+	−	−
Oct. 11	+	+	+	+	+	+	+	−	−
Oct. 17	+	+	+	+	+	+	P	−	−
Oct. 28	+	+	+	+	+	+	VS	−	−
Nov. 6	+	+	+	+	+	−	−	−	−
PIG 2, INJECTED OCT. 9, 1933, WITH 30 CC NORMAL HORSE SERUM									
Oct. 9 [4]	−	−	VS	−	−	−	−	−	−
Oct. 10	−	−	P	−	−	−	−	−	−
Oct. 11	−	−	S	−	−	−	−	−	−

[1] Key: +=complete agglutination; P=partial agglutination; S=slight agglutination; VS=very slight agglutination; −=no agglutination.
[2] Plate tests were made on same date blood was drawn.
[3] All tube tests were made on Nov. 7 with the same lot of antigen.
[4] Sample was taken before injection of serum.

The injection of the anti-swine-erysipelas serum into pig 1 produced agglutinins detectable 24 hours later by both the rapid whole-blood plate test and the tube test. The serum reacted to the tube test in a dilution of 1 to 1,000, 24 hours after the injection. The titer gradually decreased from this point, but agglutinations in a dilution of 1 to 100 were still in evidence after 1 month. The whole-blood plate test was still positive at this time, but the reaction was not so pronounced and did not develop so rapidly, although it was well marked, within 2 minutes. The injection of the normal horse serum into pig 2 failed to cause any agglutinin response.

In tests of the agglutination titer of the anti-swine-erysipelas serum (horse) injected into pig 1, positive reactions were obtained at dilutions of from 1 to 25 to 1 to 5,000. The serum was negative, however, in a dilution of 1 to 10,000. In similar tests of the normal horse serum used in the injection of pig 2, a dilution of 1 to 25 resulted in a positive reaction, all higher dilutions being negative.

To calculate data on the transfer of the specific agglutinins from the serum of one animal to that of another, the following procedure was carried out, with the results given.

Pig 2, which was injected with normal horse serum and which, as previously stated, was of about the same age and weight as pig 1, was bled out as completely as possible, to determine the approximate quantity of blood in pig 1 and the approximate quantity of serum that could be obtained from that animal. The total quantity of blood collected was 440 cc, from which 200 cc of serum was obtained.

Pig 1 had been injected subcutaneously with 30 cc of anti-swine-erysipelas serum with a titer of between 1 to 5,000 and 1 to 10,000. This specific serum could thus be considered to have been diluted about seven times. After the injection of the specific serum, the titer of the pig serum, which was between 1 to 1,000 and 1 to 2,000, would indicate that certainly a large portion of the agglutinins contained in the specific serum had been transferred to the blood of the normal pig injected.

AGGLUTINATION TESTS WITH HUMAN SERUM

In the course of experimental work on the virulence of *Erysipelothrix rhusiopathiae* for rabbits and other animals, a research worker in one of the State institutions developed an infection on the hand following direct contact of the hand with a skinned carcass of a rabbit dead after inoculation with *E. rhusiopathiae*. The first symptoms were noted July 30, 1932, in a finger 3 days after contact with the rabbit carcass. There was swelling, which later extended to other digits as well as to the hand itself, with a discoloration of the skin. The member became painful and there was a general systemic reaction. The condition persisted for some months and finally cleared up under local antiseptic treatment.

There had been no opportunity to attempt to recover *Erysipelothrix rhusiopathiae* from the patient. The diagnosis of erysipelas infection rested on the clinical symptoms and history of the case. However, samples of blood serum were collected August 31, September 24, and December 16, 1932, and the agglutination test for swine erysipelas was made. The results of the test are given in table 3.

TABLE 3.—*Results of agglutination tests* [1] *for swine erysipelas on samples of human blood serum*

[Date of exposure, July 27, 1932]

Date of collecting sample	Results of plate test of blood serum	Results of tube test of blood serum at dilutions of—						
		1:25	1:50	1:100	1:200	1:500	1:1,000	1:2,000
Aug. 31	+	+	+	+	+	+	+	−
Sept. 24	+	+	+	+	+	+	+	−
Dec. 16	−	−	−	−	−	−	−	−

[1] +=complete agglutination; −=no agglutination.

To check the specificity of these reactions, 10 samples of serum from apparently normal individuals were tested by both the plate and tube agglutination at the same time and against the same antigen. All 10 samples gave negative results to the plate test, and 7 were entirely negative to the tube test. Three of the samples gave some evidence of agglutination in the tube test at dilutions of 1 to 25 and 1 to 50.

Although no bacteriological confirmation of the diagnosis of infection with *Erysipelothrix rhusiopathiae* in this case was obtained, the history, clinical symptoms, and results of the serological tests leave little doubt as to the correctness of the diagnosis. The agglutination reaction parallels rather closely the results obtained in certain types of infection in swine.

AGGLUTINATION PLATE TEST WITH WHOLE BLOOD OF MAN HAVING CHRONIC ARTHRITIS

Since chronic arthritis is frequently a sequel of infection with *Erysipelothrix rhusiopathiae* in swine and the serums of animals so affected give strong positive reactions to the agglutination test for swine erysipelas for considerable periods, and since man is known to be susceptible to infection with this organism, a rapid, whole-blood agglutination test was made on the blood of a veterinarian with a chronic polyarthritis of some years' standing. The results of this test, however, were entirely negative.

RAPID SEROLOGICAL METHOD OF IDENTIFYING CULTURES OF ERYSIPELOTHRIX RHUSIOPATHIAE

In determining the biological characteristics of cultures of *Erysipelothrix rhusiopathiae* obtained from suspected cases of swine erysipelas or received at the laboratory for identification, the cultures are always subjected to the agglutination test against known positive swine erysipelas serum.

The usual method of preparing an antigen from cultures of the erysipelas organism for the serological test requires a considerable amount of work and time. The following is a simple method of procedure in testing cultures, which is timesaving and which has given entirely satisfactory results in the writers' identification of the erysipelas organism.

A transfer of the culture to be studied is made from a fresh broth culture to a serum agar slant, which is incubated for 24 hours. The culture is then washed off the agar slant with ½ to 1 cc of sterile normal salt solution. The quantity of salt solution used must be limited in order that the suspension may be sufficiently heavy for a satisfactory plate test. The entire growth of the organism is removed from the surface of the agar by means of a platinum loop and thoroughly suspended in the small quantity of salt solution at the bottom of the agar slant.

The plate and other equipment should now be made ready for the rapid test.

A 1 cc pipette is used for removing the suspension of organisms from the culture tube. Before the pipette is inserted in the culture tube a small, thin pledget of cotton should be folded over the tip of the pipette and securely wrapped around it to prevent the entrance of clumps of organisms, which would interfere with the proper interpretation of the test. After the pipette, containing a small quantity of the suspension, is lifted from the culture tube, the cotton is carefully removed from the tip with forceps, while the finger is retained on the upper end of the pipette to prevent the outflow of the suspension.

Two drops of the suspension of organisms from the pipette are placed on the plate for testing, and one standard loopful of hyperimmune anti-swine-erysipelas serum is added. If the organism is *Erysipelothrix rhusiopathiae*, a marked rapid clumping will take place. Organisms other than *E. rhusiopathiae* fail to cause any agglutination.

SUMMARY

Eight pigs were exposed to swine-erysipelas infection by injections of cultures of *Erysipelothrix rhusiopathiae*. Six of the pigs which showed clinical symptoms of swine erysipelas developed agglutinins in the blood detectable from the third to the sixth day after inoculation, thus indicating that the agglutinins make their appearance early in the disease, or within a brief period after the first clinical evidence of the disease is noted. The other two pigs, 3 weeks of age, failed to show any clinical evidence of infection, and the blood of these pigs remained consistently negative to all plate and tube tests for swine erysipelas, made on various dates subsequent to the time of exposures.

Blood serum from a pig which showed typical diamond skin leisons, or the urticarial form of swine erysipelas, and from which *Erysipelothrix rhusiopathiae* was recovered, gave negative results to both the plate and tube agglutination tests for swine erysipelas. Negative serological results have been reported in five other cases of typical diamond skin disease. The reason for the negative serological results in these cases has not been determined.

It has been experimentally demonstrated that the subcutaneous injection of 30 cc of anti-swine-erysipelas serum (equine) resulted in the passive transfer of agglutinins, which could be readily demonstrated within 24 hours by the plate and tube tests. The titer of the serum of the experimental pig, which was entirely negative prior to inoculation, indicates that a very large portion of the agglutinins in the specific serum had been transferred to the normal pig injected. Another pig, of approximately the same age and weight, injected

subcutaneously with 30 cc of normal horse serum remained consistently negative to both the plate and tube tests.

Blood serum from a human case, in which the history and clinical symtoms strongly indicated *Erysipelothrix rhusiopathiae* infection, gave positive reactions to both the plate and tube tests, the latter showing a titer of 1 to 1,000. After 4½ months agglutinins had apparently disappeared from this serum. A number of human sera from apparently normal individuals gave negative results to the test.

A rapid whole-blood plate test made in the case of a man affected with chronic polyarthritis gave negative results.

A description is given of the technic of a rapid serological method of identifying cultures of *Erysipelothrix rhusiopathiae*.

HISTOLOGICAL STUDIES OF RICE LEAVES INFECTED WITH HELMINTHOSPORIUM ORYZAE [1]

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INTRODUCTION

The fungus causing helminthosporium leaf spot of rice (*Oryza sativa* L.) was named *Helminthosporium oryzae* by Brede de Haan (*1*)[2] in 1900. According to Nisikado and Miyake (*6, p. 134*): "The first authentic report of the helminthosporiose of rice plant is that of S. Hori [(*4*)], who in 1892 found the disease on the glumes of rice in the suburbs of Tokyo." Of subsequent contributions to the knowledge of this disease and the causal fungus the Japanese have made by far the greatest number.

In 1924 Ocfemia (*7, 8*) reported on the occurrence of the helminthosporium disease of rice in the southern part of the United States and the Philippine Islands and investigated the relation of soil temperature to germination of certain rice varieties and to infection by *Helminthosporium oryzae*. In 1927 Ito and Kuribayashi (*5*) found the ascigerous stage of the fungus in culture and described it as *Ophiobolus miyabeanus*, but they did not find it under natural conditions. In 1928 the writer found on naturally infected rice plants in the vicinity of Crowley, La., an ascomycetous fungus that apparently is morphologically identical with the ascigerous stage of *O. miyabeanus* as described by Ito and Kuribayashi. However, these ascospores, grown on artificial culture media, have not as yet produced *H. oryzae*, nor has *H. oryzae* produced the ascigerous stage in culture for the writer.

Except for minor differences, the manifestations of the disease as it occurs in the United States seem to be the same as elsewhere in the world. The lesions produced by the fungus are usually narrowly elliptical spots with grayish centers and brown margins. Various gradations are found from narrowly elliptical lesions in some varieties to circular spots in others. Typical leaf spots are shown in figure 1.

The fungus has a number of hosts. Suematsu and Okapa (*9*) reported 39 members of the grass family and Nisikado and Miyake (*6*) 25, some of which are the same as those listed by Suematsu and Okapa.

Many ways to control the disease have been suggested, but the most satisfactory, it seems, is the development of resistant varieties. In Japan several varieties have been reported as very resistant to the disease, and the writer has noted a wide range in the resistance of commercial varieties now grown in the lower Mississippi Valley. In the United States commercial varieties have been crossed with resistant varieties from other countries. It is hoped that resistant strains of commercial value may be isolated from these crosses.

[1] Received for publication Oct. 1, 1934; issued April 1935. Investigations conducted in cooperation with the Arkansas, Louisiana, and Texas Agricultural Experiment Stations.

[2] Reference is made by number (italic) to Literature Cited, p. 90.

The purpose of the present study was to determine the nature of resistance and susceptibility to helminthosporium leaf spot in rice varieties.

MATERIAL AND METHODS

Leaves of 12 varieties of rice infected naturally in the field were used in this study. These were the short-grain varieties Kameji, Butte, Bozu, and Aikoku; the medium-grain varieties Blue Rose, Early Prolific, and Shoemed; and the long-grain varieties Storm Proof, Honduras, Fortuna, Lady Wright, and a selection from a Patna rice, J–131. Of these, Kameji, Aikoku, Butte, and Shoemed show considerable resistance and Fortuna some resistance to the helminthosporium leaf spot. The other varieties named are susceptible.

The material, with the exception of J–131, was killed and fixed in the field with strong chromo-acetic fixative.[3] The specimens were

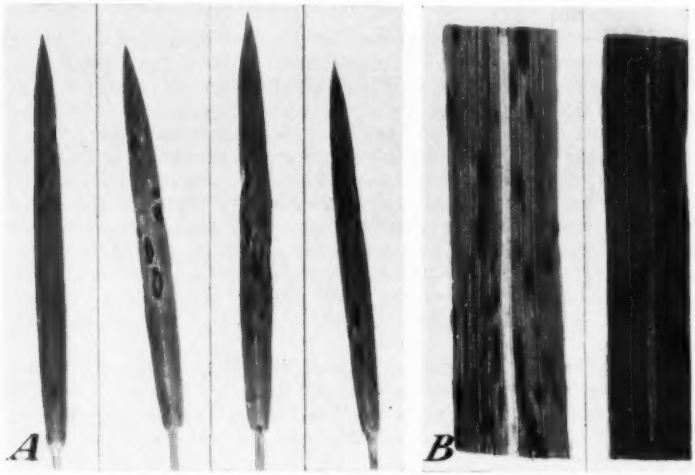

FIGURE 1.—Leaves of Blue Rose rice showing lesions caused by *Helminthosporium oryzae*. *A*, From artificial inoculations in greenhouse. × 0.85. *B*, From natural infections, Beaumont, Tex. × 1¼.

allowed to remain in the killing solution from 24 to 36 hours before being washed. As soon as the killing solution was washed out, the specimens were treated for 20 to 30 minutes with concentrated hydrofluoric acid to remove the silicon from the epidermis, and again washed.

The specimens were then dehydrated in ethyl alcohol up to 70 percent, after which a modification of Zirkle's (*10*) butyl alcohol-ethyl alcohol series was used as follows: From 70-percent ethyl alcohol the specimens were put into a 25-percent solution of *n*-butyl alcohol in 95-percent ethyl alcohol. After 2 hours this was drained off and a solution of 50-percent *n*-butyl alcohol in 95-percent ethyl alcohol was added, and 2 hours later 75-percent *n*-butyl in 95-percent ethyl alcohol. The specimens were allowed to remain in this solution overnight and were then changed to *n*-butyl alcohol. The remainder

[3] Chromic acid 1 g, acetic acid 1 g, water 100 cc.

of the schedule is the same as suggested by Zirkle (*10*). The specimens were then embedded and cut. The cross sections were cut 3μ or 6μ and the longitudinal and tangential [4] sections were cut 10μ. Sectioned material was stained in iron-alum haematoxylin and methylene blue. The former stain also was used for portions of leaves stained in toto.

ANATOMY OF HEALTHY LEAVES

The salient features of the anatomy of a rice leaf are shown diagrammatically in figure 2. The parenchyma consists of the armed-type cells referred to by Haberlandt (*3, pp. 277–278*) in *Bambusa*, *Arundinaria*, *Elymus*, *Calamagrostis*, and *Alistromeria*. This same type of

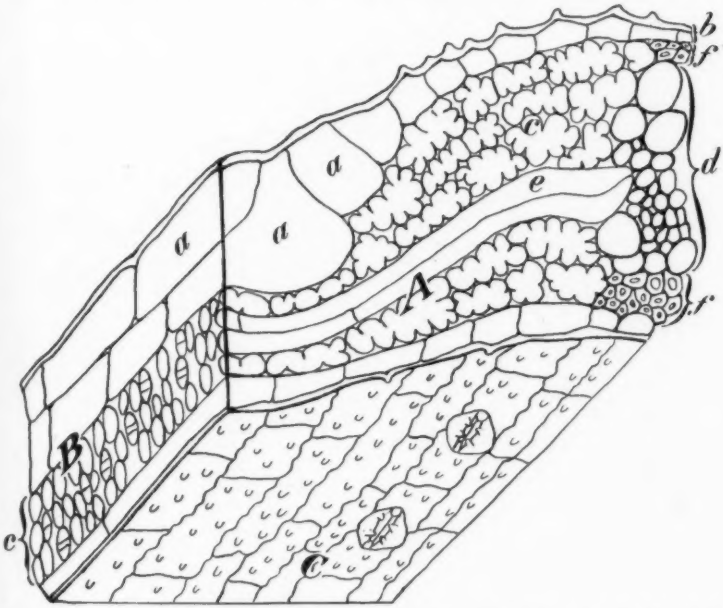

FIGURE 2.—Diagram of a portion of a rice leaf showing (*A*) cross and (*B*) longitudinal section and (*C*) surface view of lower epidermis: *a*, Motor cells; *b*, upper epidermis with papillae; *c*, armed-type parenchyma cells; *d*, fibro-vascular bundle; *e*, transverse bundle; *f*, sclerenchyma cap. × 560.

parenchyma was also found by the writer in *Arundinaria gigantea* (Walt.) Chapm. As shown in figure 2, these parenchyma cells in most cases lie with their long axes crosswise of the leaf.

In cross sections of the leaf the armed-type parenchyma cells are, in general, irregularly rectangular in outline with invaginations at the periphery. In this view their short axes range from 7μ to 15μ and their long axes from 15μ to 40μ (figs. 2, 3, *A*, *B*).

In longitudinal sections of the leaf the armed-type parenchyma cells are rectangular to elliptical in outline, depending on the portion of the cell cut and the location of adjacent cells. In this view their

[4] Longitudinal section, as here used, denotes a section cut through the leaf at right angles to its surface and parallel to its long axis; tangential section, one cut parallel to its surface.

short axes range from 4μ to 10μ and their long axes from 8μ to 20μ. In cases where the cells are cut near the periphery and through the

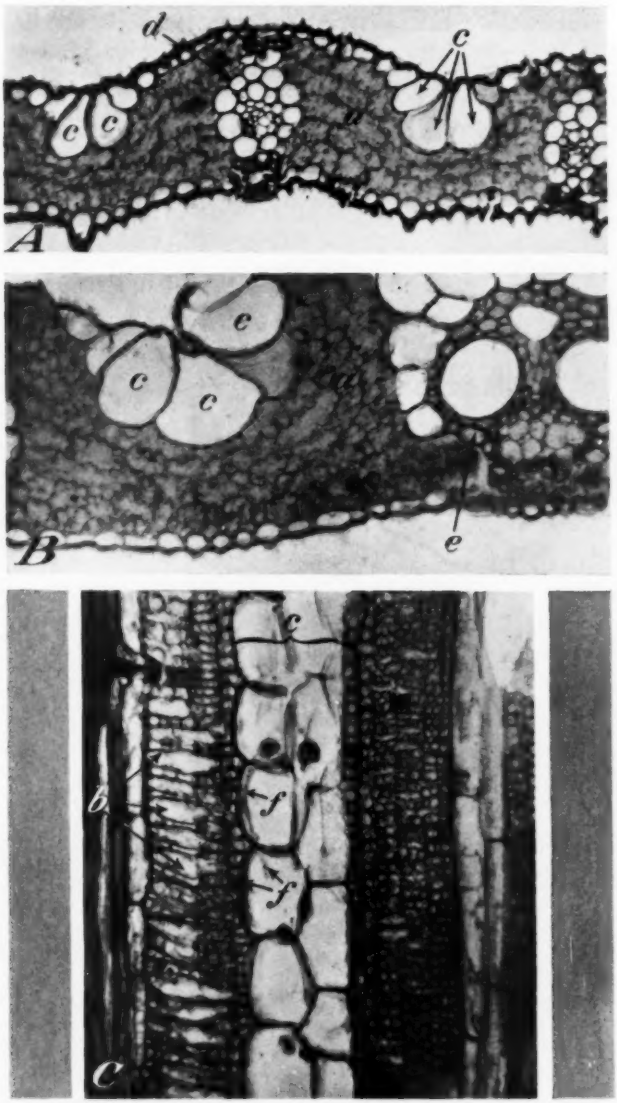

FIGURE 3.—Cross section of portion of leaf of (*A*) Shoemed and (*B*) Blue Rose rice, and (*C*) tangential section of portion of leaf of Blue Rose rice: *a*, Armed-type parenchyma cells; *b*, intercellular spaces; *c*, motor cells; *d*, small bundle; *e*, large bundle; *f*, hyphae of *Helminthosporium oryzae*. All × 288.

invaginations there appear to be numerous small cells. Examination of cross sections, however, shows that what appear to be cross walls

of small cells (fig. 3, *C*, *a*) are the invaginations of relatively large cells shown in cross sections in figure 3, *A*, *B*.

In cross sections of the leaves, as shown in figure 3, *A*, *B*, there are no conspicuous intercellular spaces. Only small openings occur

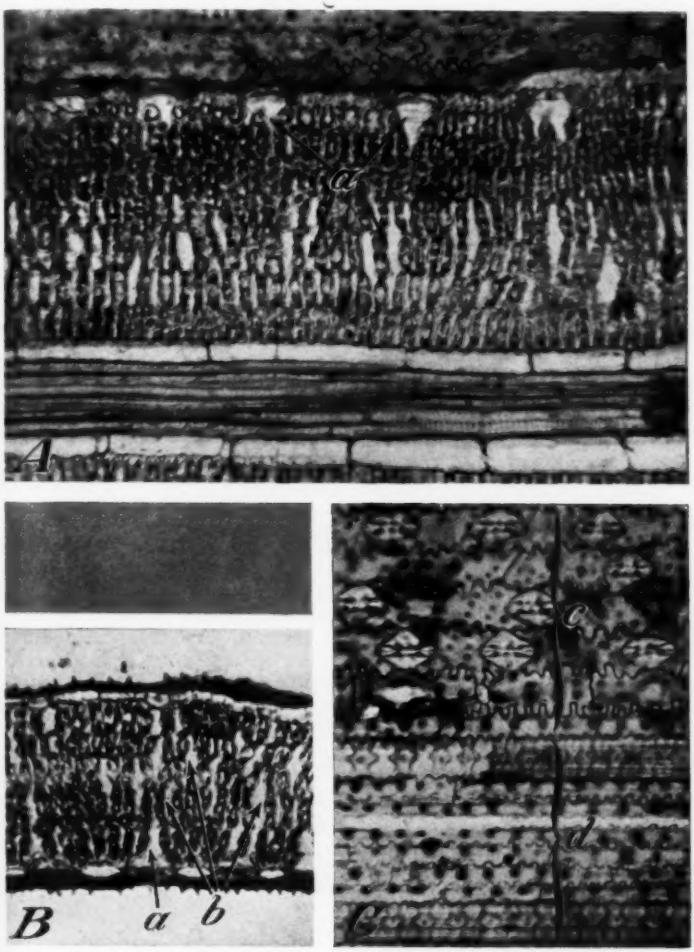

FIGURE 4.—Tangential (*A*) and longitudinal (*B*) sections of portions of leaves of Shoemed and Blue Rose rice, respectively, showing substomatal chambers (*a*) and intercellular spaces (*b*); and surface view (*C*) of portion of leaf of Shoemed rice showing lower epidermis covering parenchyma (*c*) and a large bundle (*d*). All × 320.

opposite the invaginations. There are, however, rather large substomatal chambers which are more evident in longitudinal and tangential sections (fig. 4, *A*).

In longitudinal and tangential sections intercellular spaces are abundant (fig. 3, *C*, *f*). Frequently they extend from the upper to the lower epidermis (fig. 4, *B*) and occur in bands on each side of the bundles.

Considerable difference was found in the size of the intercellular spaces of various rice varieties when examined in longitudinal sections. In Aikoku the intercellular spaces are small and relatively infrequent. In Kameji, Bozu, and Butte they are somewhat larger and occur more often. In Shoemed, Blue Rose, Early Prolific, Storm Proof, and J–131 they are still larger, and the largest were found in Fortuna and Honduras.

The stomata occur in parallel rows on the upper and lower surfaces of the leaf. The rows of stomata alternate with the bundles and motor cells on the upper surface. The bands of stomata on the lower surface (fig. 4, *C*, *d*) are opposite those on the upper surface.

The substomatal chambers are relatively large and connect with the intercellular spaces just referred to and, together with the stomata, constitute the system for the exchange of gases in the photosynthetic area of the leaf.

Most of the epidermal cells and the guard cells of the stomata are studded with papillae as shown in figures 2; 3, *A* and *B*; 4, *A* to *C*; 5, *A* and *E*; and 6, *A* to *C*. These papillae differ considerably in size, shape, and distribution as shown in the illustrations. Those on the epidermal cells extend approximately at right angles to the epidermal surface, while those on the guard cells of the stomata are inclined toward the stomatal opening. On the upper and lower epidermis there occur several kinds of hairs.

ANATOMY OF DISEASED LEAVES

The symptoms of helminthosporium leaf spot of rice, as it has been observed by the writer, coincide with the descriptions of previous investigators. Lesions have been found on plants 18 hours after inoculation, as reported by Farneti (*2*) and others. In the lesions that are just appearing, the cells in the vicinity of the invading mycelium are brownish in color and the cell walls have partly collapsed. The invading mycelium from an appressorium may enter the leaf through a stoma or penetrate directly into the cells of the epidermis, as reported by Nisikado and Miyake (*6*) and Ocfemia (*7*, *8*). It appears from the specimens examined that invasion is most frequent in the motor cells, and often the invaded motor cells are almost filled with the mycelium of the fungus. Such a condition is shown in figure 5, *A*, *B*, on a leaf of Kameji and Bozu, respectively. Hyphae in the motor cells are shown in figure 5, *A* to *C*. The section shown in figure 5, *C*, is from a portion of a leaf of Lady Wright inoculated in the greenhouse. This portion of the leaf was stained in toto to show how the hyphae have invaded the motor cells. Toxic substances produced by the fungus soon cause discoloration and death of the neighboring armed-type parenchyma cells.

Lateral branches of the mycelium that penetrate the inner walls of the motor cells are produced, and in this way the mycelium gains entrance into the intercellular spaces of the photosynthetic area of the leaf. Two systems of mycelium are then produced. One develops just beneath the epidermis and spreads through substomatal cavities

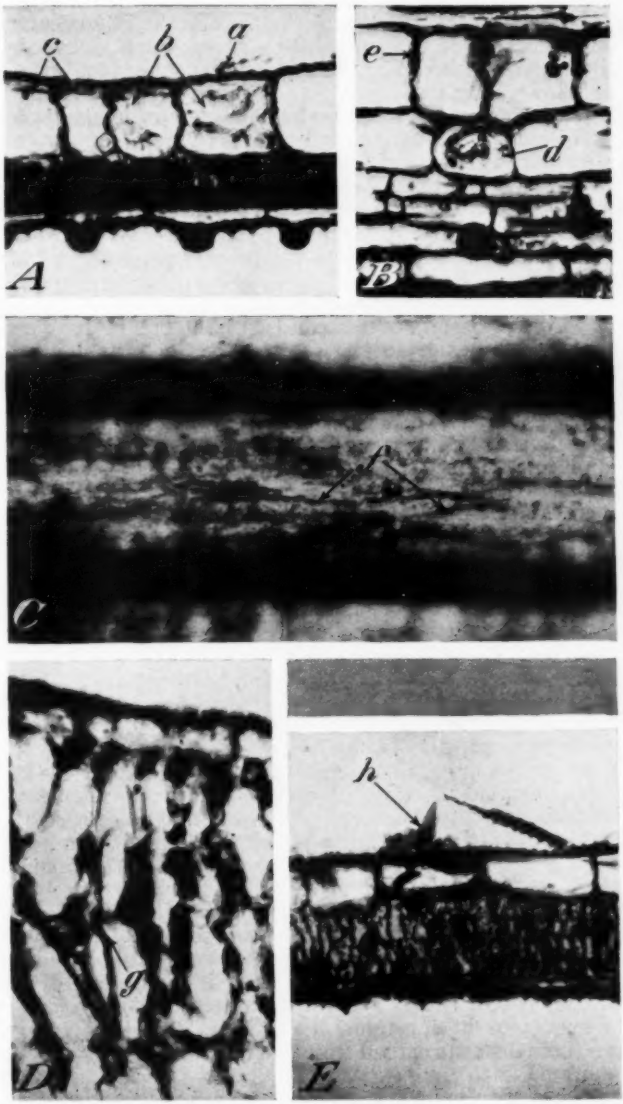

FIGURE 5.—*A*, Longitudinal section of a portion of a leaf of Kameji rice, showing remains of spore on surface of motor cell at *a*, two motor cells nearly filled with mycelium at *b*, and intracellular hypha at *c*. × 288. *B*, Tangential section of a portion of a leaf of Bozu rice, showing a cell almost filled with mycelium of the fungus at *d*, and intracellular hypha at *e*. × 288. *C*, Surface view of a portion of a leaf of Lady Wright rice, showing hyphae of the fungus in the motor cells at *f*. × 228. *D*, Longitudinal section of a portion of a leaf of Storm Proof rice, showing an intercellular hypha at *g* in an intercellular space. × 900. *E*, Longitudinal section of a portion of a leaf of Blue Rose rice, showing conidiophores, at *h*, beginning to form on an infected motor cell. × 288.

from one system of intercellular spaces to the next. The other consists of branches from this system that invade the intercellular spaces between the faces of the armed-type parenchyma cells. A lateral strand of mycelium invading the intercellular space of a leaf of Storm Proof is shown in figure 5, *D*, *g*.

The most susceptible varieties have large intercellular spaces and large substomatal chambers, invasion progresses rapidly, and the leaf spots frequently extend from the midrib of the leaf to the margin. The spots are at times circular but more often narrowly elliptical. In many cases several lesions coalesce to produce large spots.

In areas of active invasion the host cells first become yellowish in color, the chloroplasts disappear, the protoplasm dies, and the cells partially collapse.

The bundles tend to form barriers against the lateral spread of the fungus. To invade adjacent areas beyond a bundle it is necessary that the fungus penetrate the bundle sheath. Apparently this is accomplished much more easily in some varieties than in others. In very resistant varieties it apparently does not occur.

In resistant varieties invasion of the motor cells occurs, but in most cases the mycelial strands are small and soon become brownish in color and thick-walled with rather sparse contents, whereas those found in susceptible varieties are not brownish in color and are large in diameter and rich in cytoplasm, indicating much greater vigor. The conidiophores are formed from a plexus of the mycelium in the intercellular spaces or in the motor cells. The latter case is shown in figure 5, *E*. Five immature conidiophores can be seen in this group.

In resistant varieties a deposit is formed in the intercellular spaces that aids in restricting the fungus to the area of primary invasion. This ability of the parenchyma cells of an invaded area to protect those of adjoining areas from invasion is apparently the greatest factor in resistance in the leaves. These deposits are found most highly developed in Shoemed, but they also have been found to some extent in susceptible varieties. In the more susceptible varieties they are formed by groups of cells but never in sufficient numbers to isolate a given area completely, as is the case in Shoemed. Changes in the walls of the armed-type parenchyma cells and in the cells of the bundle sheath, in the immediate vicinity of the mycelium are seen in sections stained with methylene blue even before the deposits are formed. The cell walls become thicker, stain more intensely with all stains used, and after a time become somewhat yellowish in color.

The deposits are formed between the armed-type parenchyma cells considerably in advance of any mycelium, discoloration, or disintegration of the host cells. The deposits are solid in the case of the very narrow intercellular spaces and are sometimes hollow in large intercellular spaces or in substomatal chambers (fig. 6.). In resistant varieties these deposits extend from the upper to the lower epidermis, from one bundle to the next, and along the bundles. In this way, openings that may exist from one set of intercellular spaces to the next are filled so completely that in case the fungus has gained entrance into the photosynthetic portion of the leaf no further internal spread is possible. These deposits also protect the bundles so that the fungus is unable to reach additional sources of food or to invade adjacent photosynthetic areas. The protoplasmic content of the

motor cells is very limited and as soon as the reserve food obtained from the area of primary invasion is exhausted the mycelium apparently dies for want of nourishment.

The chemical nature of the deposits is not known. In unstained sections they are yellowish brown. They stain intensely with iron-alum haematoxylin, as does the outer wall of the epidermis. When they were tested for cutin, suberin, or pectin, no positive reaction was secured. Because of the high silicon content of the epidermis it is necessary to treat all material with hydrofluoric acid before sectioning. This treatment may so alter the deposits that it is not possible to determine their composition in sectioned material.

Usually the formation of the deposits is first observed between the parenchyma cells near the bundles just under the upper and lower epidermis.

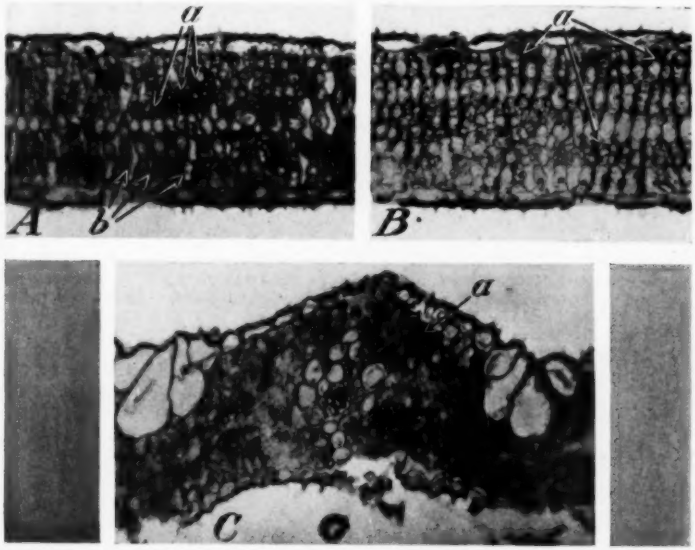

FIGURE 6.—Longitudinal (*A* and *B*) and cross (*C*) sections of portions of infected leaves of Shoemed rice, showing substomatal chambers and intercellular spaces filled with deposit at *a*. In the large intercellular spaces shown at *b* the deposits are hollow. All × 288.

SUMMARY

The anatomy of healthy rice leaves is characterized by papillate epidermis, motor cells, armed-type parenchyma cells, and usually by large intercellular spaces in photosynthetic areas.

In rice leaves affected with leaf spot caused by *Helminthosporium oryzae* the hyphae of the fungus grow intercellularly in the photosynthetic areas and intracellularly in the motor cells and bundle sheath.

The bundle sheaths of resistant varieties are less readily penetrated by the fungus than are those of susceptible varieties. The bundles interfere with the lateral spread of the fungus in the leaf.

In resistant varieties of rice the invading fungus is hemmed in by the formation of deposits, which accumulate in the intercellular spaces about an infection. The chemical nature of these deposits has not been determined.

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GNOMONIA NERVISEDA, THE PERFECT STAGE OF THE FUNGUS THAT CAUSES THE VEIN SPOT DISEASE OF PECAN FOLIAGE[1]

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INTRODUCTION

The vein spot disease of the pecan (*Hicoria pecan* (Marsh.) Britton)[3] was collected by Demaree[4] near Austin, Tex., in 1920 and again at Ferriday, La., in 1929. The writer has collected the disease on both wild and cultivated pecans in Arkansas, Louisiana, Mississippi, and Texas.

THE DISEASE

In the vicinity of Shreveport, La., vein spot first appears in May or June on the foliage of pecan trees, and in some orchards it causes the leaves of certain varieties to shed prematurely. The disease may attack the rachis, the petiole, or the veins, but the entrance of the fungus is always confined to the vascular system of the leaf. Infection may take place at or near the end of the midrib of a leaflet and extend down the midrib to its base, producing a narrow black necrotic streak seldom extending more than 2 or 3 mm on either side of the midrib. The infection may also extend from the main vascular bundle that runs lengthwise to the smaller lateral ones. When numerous small lateral bundles are killed, the leaf tissue between them dies, forming large dead areas that may later involve the entire leaflet, causing it to drop.

The fungus may attack either the terminal or the basal portion of the rachis and gradually extend to the opposite end, causing the leaflets to fall one at a time until finally the rachis, devoid of leaflets, is left hanging on the tree. In other instances, when either primary or secondary veins become infected, the fungus does not spread extensively but causes a small oval-to-circular black spot. The leaflet may have 8 or 10 of these black spots, which sometimes are not over 2 mm in diameter. The vein divides such spots into two almost equal parts. In some instances, the fungus attacks the midrib of one leaflet and causes it to abscise without interfering with the rest of the leaf, whereas in other instances it will attack the petiole and affect the entire compound leaf. In serious cases, the entire vascular system of many leaves is attacked and severe defoliation results.

Because of its striking similarity to pecan scab (*Cladosporium effusum* (Wint.) Demaree), vein spot has perhaps been confused with scab by growers and phytopathologists. Since scab spots no longer

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[2] The writer wishes to acknowledge the helpful services of the following members of the Bureau of Plant Industry: J. B. Demaree, for his criticism of the manuscript; C. L. Shear, for suggestions in the determination of the causal organism of the vein spot fungus; and Edith K. Cash, for assistance in translating the technical description into Latin.

[3] *Carya pecan* Engl. and Graebn.

[4] This information was furnished the writer through correspondence with J. B. Demaree, pathologist, Division of Fruit and Vegetable Crops and Diseases.

sporulating are black and are about the same shape and size as lesions of the vein spot disease and since scab spots also appear on the leaf veins, it is difficult by casual examination to differentiate between scab and vein spot lesions.

THE PATHOGENE

CONIDIAL STAGE

The conidial stage of the fungus causing the vein spot disease does not appear until the summer or early fall in the vicinity of Shreveport, La. The pycnidial bodies are usually most abundant about the middle of October. Immediately before the formation of the fruiting bodies, which are considered as dimidiate pycnidia, the diseased areas on the ventral side of the leaf take on a grayish color (fig. 1, *A*, *B*, *C*). The pycnidia usually appear in these areas, either singly or in groups, and are difficult to see without some magnification.

In 1933 the writer[5] described the pycnidial stage of the fungus causing the vein spot disease as *Leptothyrium nervisedum*, n. sp. It was pointed out that leaves containing the pycnidial stage of the fungus were collected in October 1930 and placed outdoors in wire cages to overwinter. The leaves were examined at regular intervals of 3 to 4 weeks to ascertain whether any development was taking place. No change was observed, but spores were found to be viable as late as May 1 the following year. It was then thought that the fungus passed the winter in the pycnidial stage. More recent investigations have demonstrated the relationship between *L. nervisedum* and a perfect stage. Pecan leaves heavily infected with the vein spot organism in 1932 were observed the following April to be bearing numerous perithecia of the *Gnomonia* type. The perithecia were found only on the past year's lesions caused by the vein spot fungus.

The work was repeated the following year with leaves collected at Ferriday and Shreveport, La., with similar results except that perithecia were less abundant that year. For reasons that will be brought out later in this paper, the fungus is considered an undescribed species and the binomial *Gnomonia nerviseda* is suggested.

TECHNICAL DESCRIPTION

Gnomonia nerviseda, n. comb.

Perithecíis plerumque hypophyllis, ellipsoideis, singulis vel gregariis, pycnidiis *Leptothyrii nervisedi* intermixtis, 250μ–275μ latis, 160μ–185μ altis, rostris 200μ–300μ longis, 75μ–100μ latis praeditis; muris peritheciorum ex strato 2–3 cellarum rufo-brunnearum vel nigrarum, muris rostrorum ex strato 5–8 cellarum similarium sed longiorum angustiorumque compositis; ascis tenuiter tunicatis, hyalinis, cylindraeceis, 36μ–42μ×8μ, apice poro munitis, ascosporis 14μ–15μ×4μ–5μ, curvatis, guttulatis, uniseptatis, ad septum constrictis, utrinque appendicula gelatinosa auctis, paraphysibus nullis.

Pycnidiis, hypophyllis, depressis-pulvinatis, 35μ–95μ latis; conidiophoris simplicibus, septatis, rectis vel leniter curvatis, 14μ–18μ longis, 2μ–4μ latis; conidiis irregularibus interdum curvatis, oblongisovatis, continuis, 8μ–13μ longis, 2μ–3μ latis.

In foliis *Hicoriae pecan* (Marsh.) Britton, Arkansas, Louisiana, Mississippi, et Texas.

Perithecia mostly hypophyllous, ellipsoidal, single or gregarious, intermingled with pycnidia of *Leptothyrium nervisedum*. The perithecia are 250μ–275μ wide by 160μ–185μ high. The beaks measure from 200μ–300μ long by 75μ–100μ wide. The walls of the perithecia are 2 to 3 cells thick while those of the beaks are 5 to 8 cells thick, but the cells of the beak are much longer and narrower than those of the perithecia. The cells of both the perithecia and beaks are reddish brown to

[5] COLE, J. R. VEIN SPOT OF THE PECAN CAUSED BY LEPTOTHYRIUM NERVISEDUM, N. SP. Jour. Agr. Research 46: 1079-1088, illus., 1933.

black in color. Asci, thin-walled, hyaline, cylindrical, 36μ–42μ by 8μ with a pore at the apical end. Ascospores, 14μ–15μ by 4μ–5μ, curved, guttulate, 1 septate, constricted at septum and with gelatinous appendages at each end. Paraphyses not present.

Pycnidia hypophyllous, depressed pulvinate, 35μ–95μ wide, conidiophores simple, septate, straight or slightly curved, 14μ–18μ by 2μ–4μ; conidia irregular, sometimes curved, oblong to ovate, hyaline, nonseptate, 8μ–13μ by 2μ–3μ.

FIGURE 1.—*A* and *C*, Pecan leaflets showing pycnidia on midrib; *B*, rachis discolored by vein spot fungus.

Lesions occur only on the vascular system of foliage of *Hicoria pecan* (Marsh.) Britton. The fungus has been found in Arkansas, Louisiana, Mississippi, and Texas.

Type specimens have been deposited in the pathological collections of the Bureau of Plant Industry, United States Department of Agriculture, Washington, D. C.

PATHOGENICITY

Ascospore cultures were made from the *Gnomonia* perithecia and the resultant growth was identical with that made from the conidia of *Leptothyrium nerviseduni* Cole. Pycnidialike bodies, containing conidia, were produced in the ascospore cultures, but no perithecia were formed.

In May 1933, pecan leaves of the Van Deman variety were inoculated with ascospores from leaves collected at Ferriday, La.; with conidia from ascospore cultures; and with conidia from pycnidia from leaves collected in 1932. The results are shown in table 1.

TABLE 1.—*Results of inoculating leaves of the Van Deman variety of pecan with conidia and ascospores of the vein spot fungus*

[50 inoculations were made for each test]

Series no. and date of inoculation	Inoculum	Infections	Date of reading	Remarks
		Number		
1 (May 10)	Conidia from leaves	37	May 30	All inoculations were made on the leaf veins; both immature and mature.
	Conidia from ascospore cultures	44		
	Ascospores from leaves	30		
	Check	2		
2 (June 8)	Conidia from leaves	29	June 28	Leaves were inoculated with no apparent difference in the susceptibility.
	Conidia from ascospore cultures	36		
	Ascospores from leaves	19		
	Check	8		
3 (July 1)	Conidia from leaves	34	July 21	
	Conidia from ascospore cultures	41		
	Ascospores from leaves	14		
	Check	10		

The vein spot disease was produced from all three series of inoculations. Infections appeared in 2 to 3 weeks after the inoculations were made, with no noticeable difference in the time required for infection to take place from ascospores or conidia.

Several species of fungi belonging to the genus *Gnomonia* have been reported on the pecan, but the spores of only two of them have appendages. Ellis and Everhart [6] described one species, *Gnomonia setacea* (Pers.) Ces. and DeNot., var. *macrospora* Ell. and Ev., occurring on fallen leaves of *Quercus*, *Castanea*, and *Carya* (*Hicoria*) near Newfield, N. J., as having setae, but an examination of the type specimens by the writer disclosed that the ascospores either were immature or else had deteriorated so much that a fair comparison could not be made. However, the perithecia of the above fungus are not confined to any part of the leaves, being scattered over the entire leaf surface, and there was no evidence of old *Leptothyrium* pycnidia among the perithecia. The description of the above organism, according to Ellis and Everhart, indicates that it is different from the *Gnomonia* associated with the vein spot fungus. They describe *G. setacea* var. *macrospora* as being 4- to 8-spored and septate in the middle without constriction. The asci of *G. nerviseda* always contain 8 spores and they show a constriction at the septum (fig. 2, *F*).

Matz [7] described a *Gnomonia* on living pecan leaves in Florida in 1917. Since the asci of this species contain usually 2 and rarely 1, 3,

[6] ELLIS J. B., and EVERHART, B. M. THE NORTH AMERICAN PYRENOMYCETES: A CONTRIBUTION TO MYCOLOGIC BOTANY. p. 326. Newfield, N. J. 1892.

[7] MATZ, J. AN UNDESCRIBED GNOMONIA ON PECAN LEAVES. Fla. Agr. Expt. Sta. Ann. Rept. 1917: 89R-94, illus. 1918.

or 4 spores, variable in size, and since it is not known to be connected with a conidial stage, it is considered by the writer as a separate species from *Gnomonia nerviseda*.

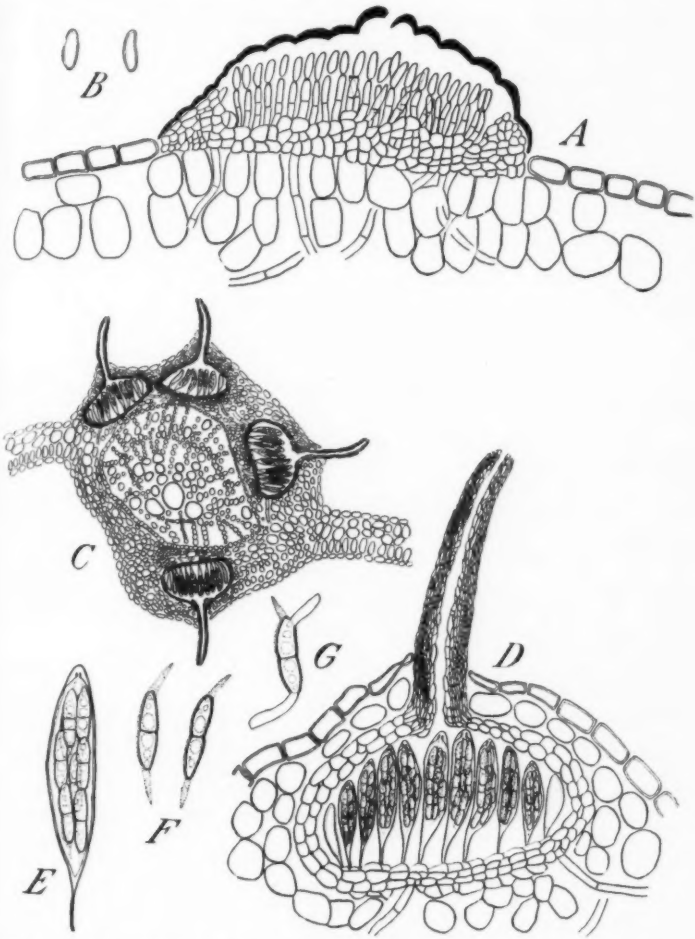

FIGURE 2.—The vein spot fungus. *A*, Cross section of a leaf with a pycnidium on the midrib, × 400. *B*, Representative conidia, × 800. *C*, Cross section of a leaf vein, in early spring, showing the perithecia on both ventral and dorsal sides of the leaflet; however, the majority are on the lower or ventral side, × 100. *D*, Typical perithecium from section of midrib, × 400. *E*, Representative ascus showing typical shape, × 900. *F*, Representative ascospores enclosed in gelatinous membrane, × 1,200. *G*, Ascospore germinating after 24 hours, × 1,200.

Wolf [8] described *Gnomonia caryae*, the perfect stage of *Leptothyrium caryae* (Ell. and Ev.) Cole (*Gloeosporium caryae* Ell. and Dearn.), on hickory leaves (*Carya ovata* (Mill.) K. Koch), but the perithecia of this fungus are scattered over the entire leaf surface with spores 25μ

[8] WOLF, F. A. A NEW GNOMONIA ON HICKORY LEAVES. Ann. Mycol. 10: 488-491, illus. 1912.

to 33μ long and 5μ wide and have no appendages, whereas the sporee of *G. nerviseda* average 14μ to 15μ long and 4μ to 5μ wide and arg enveloped in a gelatinous substance drawn out to a point resemblins setae.

Cole [9] described *Gnomonia caryae* var. *pecanae*, nov. var., on the pecan (*Hicoria pecan*), but this fungus is morphologically different, both microscopically and macroscopically, from *G. nerviseda*. The spores of both species bear gelatinous appendages, but those of *G. caryae* var. *pecanae* are 22μ to 28μ long and 3μ to 5μ wide and perithecia measure 300μ to 350μ wide and 150μ to 250μ high, excluding the beaks. The spores of *G. nerviseda* are 14μ to 15μ long and 4μ to 5μ wide, with perithecia 250μ to 275μ wide and 160μ to 185μ high, excluding the beaks. Conidia from ascospore cultures of *G. caryae* var. *pecanae* produced lesions typical of that formed by the conidial stage of the liver spot fungus, *Leptothyrium caryae* var. *pecanae*, while conidia from ascospore cultures of *G. nerviseda* produced lesions typical of the conidial stage of the vein spot fungus, *L. nervisedum*.

SUMMARY

Vein spot, a foliage disease of pecans, *Hicoria pecan* (Marsh.) Britton (*Caryae pecan* Engl. and Graebn.), caused by *Gnomonia nerviseda*, n. comb., has been collected by the writer in Arkansas, Louisiana, Mississippi, and Texas.

The disease attacks the rachis, the petiole, or the veins, and is always confined to the vascular system of the leaf, thereby suggesting the name "vein spot." Heavy infection results in severe injury to the foliage or in premature defoliation.

The pycnidial stage of the vein spot fungus, *Leptothyrium nervisedum* Cole, does not appear until summer or early fall, while the perfect stage appears the following spring on the fallen leaves along the old vein spot lesions.

The writer was able to demonstrate the relationship between the pycnidial and the perfect stage of the vein spot fungus.

The perfect stage of the vein spot fungus is described as new, *Gnomonia nerviseda*, distinct from *G. setacea* var. *macrospora* Ell. and Ev., from a two-spored *Gnomonia* described by Matz in Florida, from *G. caryae* Wolf, and from *G. caryae* var. *pecanae* Cole.

[9] COLE, J. R. LIVER SPOT DISEASE OF PECAN FOLIAGE CAUSED BY GNOMONIA CARYAE PECANAE, NOV. VAR. Jour. Agr. Research 47: 869-881, illus. 1933.

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